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Printed in Canada by: The Runge Press Limited, Ottawa

Imprimé au Canada par: The Runge Press Limited, Ottawa

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979.

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 45, No. 12, December 1988 | **Volume 45, nº 12, décembre 1988**

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Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, Oceanic Abstracts, and Science Citation Index

Area of Attraction and Effective Area Fished by a Whelk (*Buccinum undatum*) Trap under Variable Conditions

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McQuinn, I. H., L. Gendron, and J. H. Himmelman. 1988. Area of attraction and effective area fished by a whelk (*Buccinum undatum*) trap under variable conditions. Can. J. Fish. Aquat. Sci. 45: 2054–2060.

A study was conducted to examine the size and shape of the area of attraction, to evaluate the effective fishing area of a whelk (*Buccinum undatum*) trap under different physical and biotic conditions, and to validate the whelk density estimates as determined from the effective area. The study was conducted using a tag–recapture method in the Archipel de Mingan, Quebec. The effect of six different variables was examined (season, bait, depth, substratum, current, and stress). Current speed and direction were shown to be important in defining the size and shape of the area of attraction. Estimates of the effective area ranged from 18 to 278 m^2. Lowest values were associated with relatively strong currents (26 $cm \cdot s^{-1}$), while highest values were observed in July and when a less stressful tagging procedure was used. Behavioural factors, notably seasonal variations in feeding rates and reproductive activity, also appeared to affect the size of the effective area. Density estimates derived from the effective areas were comparable with SCUBA diving survey estimates. Our study shows that the effective area cannot be estimated as a single, gear-specific value. Effective area estimates must therefore be determined on a site- and season-specific basis if conditions affecting the catchability of whelks vary during the fishing season and/or over the fishing area.

La présente étude a été menée dans le but d'examiner la taille et la forme de l'aire d'attraction et d'évaluer l'aire effective de pêche d'un casier à buccins (*Buccinum undatum*) selon différentes conditions physiques et biotiques. Cette étude visait aussi à valider les estimations de densité obtenues à partir du calcul de l'aire effective de pêche. L'étude a été menée autour de l'Archipel de Mingan (Québec) à partir de buccins marqués et l'effet de six différentes variables a été examiné (saison, appât, profondeur, substrat, courant et stress). La direction et la vitesse du courant sont apparues importantes dans le détermination de la forme et de la taille de l'aire d'attraction. Les estimations de l'aire effective de pêche ont montré des variations allant de 18 à 278 m^2. Les plus faibles valeurs ont été associées avec des conditions de forts courants (26 $cm \cdot s^{-1}$) alors que les plus fortes valeurs ont été observées en juillet et lorsqu'une technique de marquage moins stressante pour les buccins a été utilisée. Des facteurs comportementaux, notamment le taux d'alimentation et l'activité reproductive sont apparus importants quant à leur effet sur la taille de l'aire effective de pêche. Les estimations de densité de buccins effectuées à partir des valeurs de l'aire effective de pêche sont comparables aux estimations de densité obtenues en plongée sous-marine. Cette étude démontre que l'aire effective de pêche d'un casier à buccins ne peut pas être restreinte à une seule et unique valeur. L'aire effective de pêche doit être déterminée pour chaque site et saison spécifiquement, si les conditions affectant la capturabilité des buccins varient au cours de la saison de pêche et/ou sur le territoire de pêche.

Received January 19, 1988
Accepted August 9, 1988
(J9549)

Reçu le 19 janvier 1988
Accepté le 9 août 1988

Common northern whelks (*Buccinum undatum*) have traditionally been exploited in small local fisheries along the east coast of Canada, and particularly in the St. Lawrence Estuary and near Havre-Saint-Pierre (Caddy et al. 1974). From 1949 to the early 1980's, landings in the Province of Quebec fluctuated between 5 and 486 $mt \cdot yr^{-1}$ depending mainly on local markets. However, with the recent increase in international market demand for whelks from the Gulf of St. Lawrence, annual landings have been increasing constantly, reaching 1200 mt in 1987.

Villemure and Lamoureux (1975) showed that whelk population structure can be drastically altered after a few years of low intensity fishing. The highly efficient traps used in this fishery make whelk populations particularly vulnerable to overexploitation. Once local whelk stocks have been depleted, their recovery can be slow due to low growth rates (Hancock 1963; Y. Boivin, Université du Québec à Chicoutimi, Chicoutimi (Québec), unpubl. data) and limited expansion from adjacent stocks due to their benthic embryonic stages and restricted adult movements (Hancock 1963). With the increasing demand for whelks, there is a clear need for effective stock management requiring density estimates and the determination of appropriate exploitation levels.

Resource assessment models based on the area fished by baited gear and designed to estimate population densities from readily available catch per unit effort data have been developed

and reported in the literature. These models involve two main concepts: that of the area of attraction and the effective area fished. The area of attraction refers to the size and shape of the area of chemical influence of a trap within which animals can detect the bait and are able to reach it within a specific period of time. The effective area fished by a trap, as introduced by Miller (1975), is defined as the theoretical area within which all individuals have a 100% probability of capture. The effective area can therefore be used directly for population density estimates.

Several models have been described for the estimation of the area of attraction of traps and baited hooks. Eggers et al. (1982) described a model whereby nonlinear least squares optimization was used to estimate the radius of the area of attraction by evaluating catches from a series of capture devices along a ground line with varying degrees of overlap between the areas of attraction. This model assumed that the area of attraction of each device was circular. Gros and Santarelli (1986) estimated the area of attraction of whelk (*Buccinum undatum*) traps using a similar model, although they assumed an elliptical shape for the area of attraction based on the shape of the M_2 tidal distributions in the study zone. Theoretically, this would be more realistic than the circular models developed previously.

Ito et al. (1981) evaluated the area of attraction of whelk (*Neptunea arthritica*) traps directly, from which whelk density was subsequently calculated (Ito and Tachizawa 1981) by releasing tagged whelks at various distances from a trap and recording their recapture rates. However, in their study, the tagged whelks were not released evenly around the trap and therefore the area of attraction was poorly defined. In addition, recapture rates were extremely low, probably because the whelks were stressed during tagging and because the tagged animals were allowed to feed prior to release.

Density estimates obtained from these models can be biased when incorrectly assuming that the area of attraction is equal to the effective area. Miller (1975) determined the effective area directly for commercial crab (*Chionoecetes opilio*) traps as the ratio of catch per trap to crab density estimated from photographs. This single value was subsequently used with catch and effort data to estimate stock densities in other areas (Greendale and Bailey 1982).

While all these methods can provide inexpensive density estimates, they are limited in both accuracy and precision by assuming that catchability and therefore the effective area are constant under all conditions. Hancock (1963) discussed several factors affecting the catchability of whelks including water temperature, breeding period, and bait type. In addition, Younker and MacWilliams (1971) noted that whelk catches varied with current strength and substratum type.

Methods of estimating the effective area fished by a trap must consider the factors which influence its size. Our main objectives were therefore to (1) examine the effects of six physical and biotic factors (season, bait, depth, substratum, current, and stress) on the size and shape of the area of attraction and the size of effective area fished around a whelk trap and (2) validate the whelk density estimates determined from the effective areas and the catch per trap by comparison with estimates from SCUBA diving surveys.

Materials and Methods

The study was conducted during the summers of 1983 and 1984 at five sites around Île du Havre in the Archipel de Mingan, northern Gulf of St. Lawrence (Fig. 1). Sites were selected so that the conditions of the different treatments would be similar to a standard treatment (treatment 2) with the exception of the single variable tested (Table 1). However, in spite of our familiarity with the study area, and considerable time spent searching for sites, it was difficult to find sites with exactly the desired conditions. For example, the site of treatment 6 was chosen to test the effects of a different substratum, but it was later found that the current speed was almost as great as on the site of treatment 7, which was chosen for its strong current. These variations were subsequently taken into account when comparing the results of the different treatments.

Experimental Procedure

Areas of attraction and effective areas were estimated by releasing tagged whelks at set distances from a baited trap and recording their recapture rates. At each site, two 100-m lead lines were laid along the bottom, one running roughly in the direction of the dominant current and the other perpendicular to it, intersecting in the middle to produce four transect lines (Fig. 2). Whelks used in the experiment were captured off Île du Fantôme in traps baited with herring. The bait was covered by a nylon wrap to prevent the whelks from feeding on it. The captured whelks were placed in buckets of seawater and transported within an hour to holding basins in a wet laboratory at Havre-Saint-Pierre. During the 2-h tagging procedure, the whelks were kept as much as possible in cool, well-oxygenated running seawater. The release point (treatment and transect line numbers and distance from the trap) of each whelk was recorded on a masking tape tag attached to an elastic band which was wound around the shell. Only whelks >60 mm in shell length (from the tip of the spire to the extreme edge of the lip) were used.

A total of 760 whelks were released immediately following tagging (<1 h) for each treatment. The whelks were transported by divers in meshed diving bags to the release positions along the transect lines shown in Fig. 2. A trap was placed at the intersection of the transect lines immediately after release and was baited with previously frozen herring (or a fresh cod head in the case of the bait treatment) weighing 150–200 g. All captured whelks (tagged or untagged) were collected by divers after 6 h (to avoid possible trap saturation) and 24 h of fishing. Shell length and sex were recorded for all captured whelks.

From our experience, and from published accounts of whelk tagging experiments (Hancock and Urquhart 1959), whelks often exhibit a period of stress-induced inactivity after release, following extended tagging manipulations. To examine to what extent the laboratory tagging procedure stressed the whelks and thus modified their reaction to the bait, divers collected 100 additional whelks during treatment 3 which were tagged at the site. These whelks were transported over a short distance and were maintained under water in nets suspended from the sides of a boat. They were never out of water for more than 15 s. Twenty and 30 individuals were placed at 2 and 10 m, respectively, along transect lines 2 and 3 next to the laboratory tagged individuals. A stress factor was estimated as the ratio of the grouped recapture rates of the laboratory tagged whelks to the corresponding field tagged individuals to correct the subsequent density estimates for differences in catchability.

To measure the effects of the laboratory tagging manipulations on the estimates of the area of attraction and the effective area, treatment 8 was conducted using whelks which were all

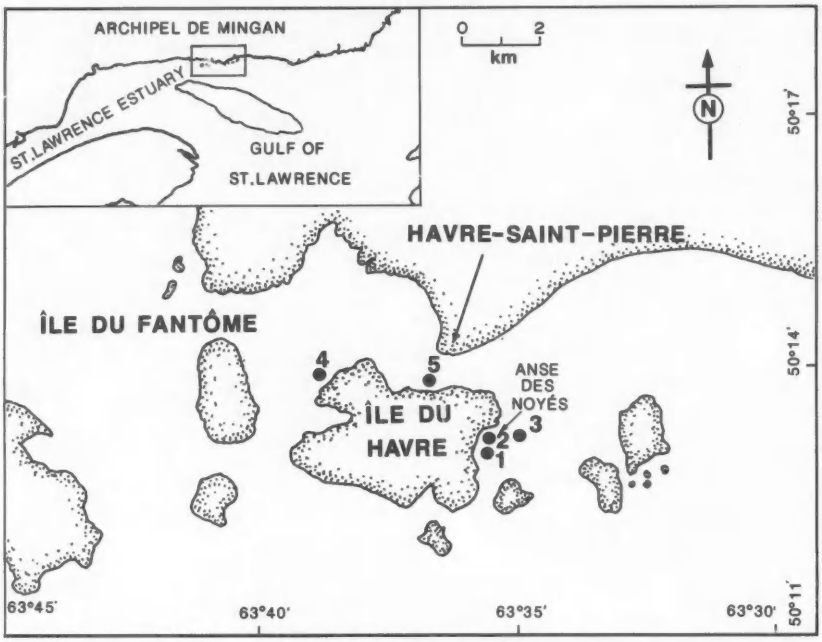

FIG. 1. Archipel de Mingan showing the five study sites.

TABLE 1. Experimental design for the study of the effects of selected factors on the effective area fished by a whelk trap. Mixed substratum = sand and gravel, scattered boulders.

Treatment	Site (No.)	Date	Bait	Depth (m)	Substratum	Current ($cm \cdot s^{-1}$), mean ± SD (n)	Tagging
1. June	Anse des Noyés (1)	2–3 June 1983	Herring	10	Mixed	11 ± 3 (917)	Laboratory
2. July	Anse des Noyés (1)	4–5 July 1983	Herring	10	Mixed	10 ± 3 (2160)	Laboratory
3. August	Anse des Noyés (1)	1–2 August 1983	Herring	10	Mixed	~10	Laboratory
4. Bait	Anse des Noyés (2)	6–7 July 1983	Cod	10	Mixed	~10	Laboratory
5. Depth	Île du Havre (east side) (3)	4–5 July 1983	Herring	20	Mixed	9 ± 6 (256)	Laboratory
6. Substratum	Île du Havre (west side) (4)	5–6 July 1983	Herring	10	Mud	21 ± 11 (2132)	Laboratory
7. Current	Channel between Île du Havre and Havre-St-Pierre (5)	7–8 July 1983	Herring	10	Mixed	26 ± 14 (2020)	Laboratory
8. Stress	Anse des Noyés (1)	1–2 August 1984	Herring	10	Mixed	~10	Field

collected by divers and tagged at the treatment site. The results of this treatment were compared with treatment 3, which was also performed in August. Treatment 8 was conducted within 100 m of the site of treatments 1, 2, and 3.

Whelks were surveyed within 1 m of each side of the transect lines by divers prior to each treatment to derive sex ratio and density estimates of the natural population. However, during the course of the summer, we noticed that whelks were often found to be almost completely buried. Often only the syphon was visible at the substratum surface. Hancock (1963) also reported this behaviour for *Buccinum* populations in England. Since we suspected that many individuals were missed during these pretreatment surveys, these data were considered unreliable for density estimation. An intensive survey of the benthos in the Archipel de Mingan (P. Jalbert, Department of Fisheries and Oceans, Mont-Joli, Que., unpubl. data) provided estimates of whelk densities for comparison with those calculated from the effective area and the catch of untagged whelks. Whelks

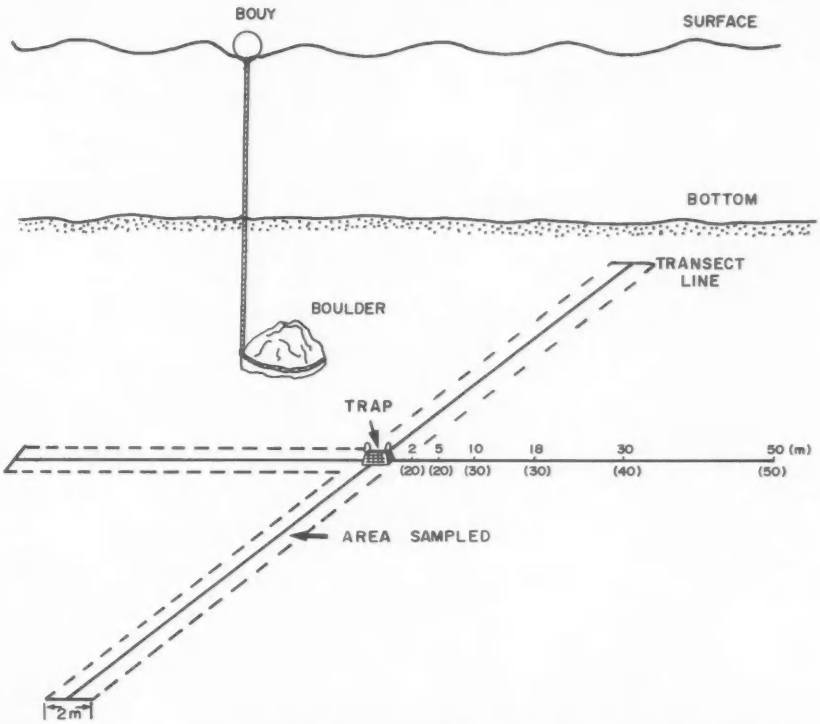

FIG. 2. Design of a treatment site showing the area sampled for the pretreatment surveys and the release distances from the trap. The number of whelks released at each distance is indicated in parentheses.

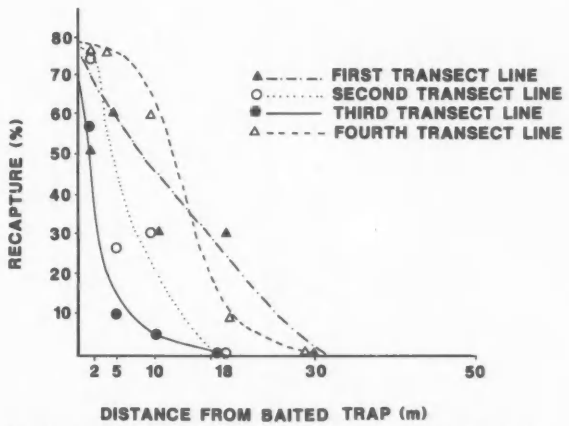

FIG. 3. Plot of percentage of whelks recaptured after 24 h of fishing in relation to the release distance from the trap for the four transect lines of treatment 8.

were censused in June and August at depths of between 8 and 12 m along transects within 500 m of our treatment sites.

Estimation of Effective Areas and Densities

Percent recapture after 24 h was plotted against release distance for each transect line of each treatment. Smoothed recapture rate curves were visually derived from these data points for each transect line, as shown in Fig. 3 for treatment 8 (stress). The estimated distances from the trap at which 2, 5, 10, 20, and up to 70% recapture was attained were determined from these curves for each transect line. The points were connected to form recapture rate isopleths around the trap for each treatment (Fig. 4). The area of each zone between the isopleths (S_i) was obtained by planimetry. The percentage recapture (R_i) assigned to each zone was defined as the mean value of the recapture rates of two successive isopleths. The zone nearest the trap was assigned the value of the innermost isopleth. The area of attraction was therefore the sum of the areas of each zone:

$$S = \sum_{1}^{i} S_i$$

and the effective area was expressed as

$$A = \sum_{1}^{i} A_i$$

where

$$A_i = R_i \cdot S_i.$$

The effective area was thus derived from the area of attraction and was expressed as a reduced, standardized area for which the recapture rate would be 100%.

Assuming a homogeneous whelk distribution, population density (D) was calculated using the relationship defined by Miller (1975):

(1) $D = C/A$

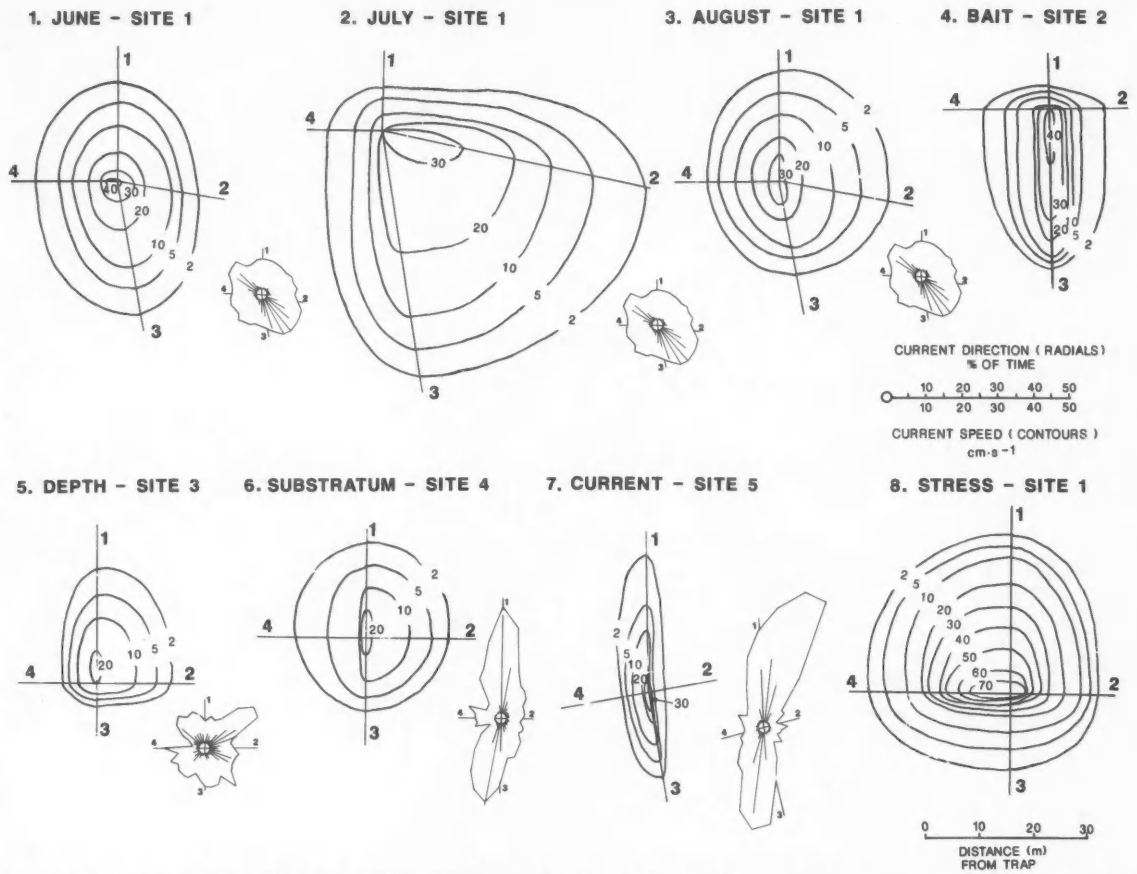
1. JUNE - SITE 1
2. JULY - SITE 1
3. AUGUST - SITE 1
4. BAIT - SITE 2
CURRENT DIRECTION (RADIALS)
% OF TIME
10 20 30 40 50
10 20 30 40 50
CURRENT SPEED (CONTOURS)
cm·s⁻¹
5. DEPTH - SITE 3
6. SUBSTRATUM - SITE 4
7. CURRENT - SITE 5
8. STRESS - SITE 1
0 10 20 30
DISTANCE (m)
FROM TRAP

FIG. 4. Recapture rate isopleths obtained after 24 h of fishing as a function of distance from the trap for each treatment, and current profiles showing frequency distributions of direction at 10° intervals (radials) and average speed in each direction (contours) at four of the treatment sites around Île du Havre. Numbers identify the four transect lines.

TABLE 2. Areas defined by the percent recapture isopleths (S_i), corresponding effective areas (A_i), area of attraction (S), and effective area (A) fished by a whelk trap for each treatment. Area expressed in m^2.

Treatment		Recapture rate (%) 3.5	7.5	15	20	25	30	35	40	45	55	65	70	Total	
1. June	S_i	349	240	246		82		24	3					S	944
(early summer)	A_i	12	18	37		21		9	1					A	98
2. July	S_i	877	650	507		411	65							S	2509
(midsummer)	A_i	31	49	76		103	19							A	278
3. August	S_i	363	275	227		84	17							S	965
(late summer)	A_i	13	21	34		21	5							A	93
4. Bait	S_i	242	112	116		57		66	13					S	606
(cod)	A_i	9	8	17		14		23	5					A	77
5. Depth	S_i	161	131	113	9									S	414
(20 m)	A_i	6	10	17	2									A	34
6. Substratum	S_i	299	230	109	11									S	648
(mud)	A_i	11	17	16	2									A	46
7. Current	S_i	140	53	47		4	4							S	247
(0.26 m·s⁻¹)	A_i	5	4	7		1	1							A	18
8. Stress	S_i	322	296	301		171		127		82	65	30	26	S	1420
(field tagged)	A_i	11	22	45		43		45		37	36	20	19	A	276

where C is the total number of untagged whelks caught after 24 h. The stress factor, derived from treatment 3, was used to correct the density estimates from all treatments which involved laboratory-tagged whelks.

Current Data

During June and July 1983, a current meter was installed approximately 2 m from the bottom and within 5 m from the centre of four of the five sites for a period of at least 2 wk, except for site 3 (treatment 5), where only a 36-h record was obtained. Current direction and speed were recorded at 10-min intervals. These data were used to produce current profiles for these sites (Fig. 4). Average current speed for the bait treatment at site 2 and the August treatments at site 1 were assumed to be similar to site 1 in June and July (Table 1).

Results

The areas encompassed by the recapture rate isopleths, the area of attraction, and the effective area showed marked variations among the treatments (Table 2). Comparison of the current direction profiles with the areas of attraction from the various sites (Fig. 4) demonstrated how current direction shaped the area of attraction. Recapture rates were notably greater along transect lines which were most frequently downstream from the trap thus elongating the isopleths in the dominant current directions. This was particularly evident in treatment 7, performed under very strong, bidirectional currents.

Estimates of the effective area ranged from 18 m^2 (treatment 7) to 278 m^2 (treatment 2). From comparisons of the effective areas of the various treatments with treatment 2 (standard), several patterns emerged. The size of the effective areas was greatly reduced in strong currents. Treatment 7, where current speed was on average twice that of treatment 2, yielded the smallest estimate (18 m^2). Strong currents likely cause a greater dilution of the attractant substances from the bait and may also reduce the rate of whelk movement upstream, thus markedly reducing the effective area. Younker and MacWilliams (1971), from studies in the Gulf of St. Lawrence, also reported that whelk catches were lower in areas of strong current conditions. In light of this obvious effect of current strength on catchability, it was impossible to determine whether the reduced effective area estimated for the substratum treatment (46 m^2) was due to the mud bottom or the current speed, which was almost as strong as on the site of the current treatment (21 versus 26 $cm \cdot s^{-1}$).

Striking seasonal variations in the effective areas were indicated by the differences among treatments 1 (98 m^2), 2 (278 m^2), and 3 (98 m^2) conducted on the same site in June, July, and August, respectively. The larger effective area obtained in treatment 2 was unexpected but could be accounted for by variations in catchability due to the reproductive state of the females and hence changes in feeding rates. Martel et al. (1986a) showed that the percentage of females actively laying egg capsules was significantly higher in July than in June and August, coinciding with a period of low feeding activity (Martel et al. 1986b). Hancock (1963) also noted seasonal changes in feeding rates of *Buccinum* along the east coast of England, which he attributed to reproductive activity. From our experiment, the percentage of females in the untagged captures of treatment 2 was significantly less (test of percentages, $p < 0.05$; Sokal and Rohlf 1969) than that observed in the natural population (17 versus 46%), indicating a lower feeding rate for these females. However, there was no difference between the percentage of tagged females released and subsequently recaptured in this treatment. The effective area was most likely overestimated due to the higher catchability of the experimental population.

TABLE 3. Effective area estimates (m^2), total untagged captures (C), and whelk densities ($no. \cdot m^{-2}$) for each treatment calculated from captures after 24 h (raw and corrected for stress factor) and estimated from intensive diving surveys.

Treatment	Effective area	C	Calculated density		Survey density
			Raw	Corrected	
1. June (early summer)	97.5	113	1.16	0.67	0.52
2. July (midsummer)	277.6	116	0.42	0.24	0.52
3. August (late summer)	93.4	84	0.90	0.52	0.52
4. Bait (cod)	76.7	86	1.12	0.65	0.52
5. Depth (20 m)	34.2	103	3.01	1.74	—
6. Substratum (mud)	46.3	20	0.43	0.25	0.58
7. Current (0.26 $m \cdot s^{-1}$)	18.0	43	2.39	1.39	1.38
8. Stress (field tagged)	276.4	178	0.64	—	0.52

The estimated effective area of treatment 5 (34 m^2), conducted at 20 m, was also much smaller than treatment 2. Here the current speed (9 $cm \cdot s^{-1}$) was similar to treatment 2 (10 $cm \cdot s^{-1}$) although the current had several predominant directions (Fig. 4). We could not discern whether the reduction of the effective area was due to depth- or current-related factors, although it is possible that whelks may have had more difficulty in locating the bait, since current was less directional than in treatment 2.

The use of cod, rather than herring, in treatment 4 also yielded an effective area estimate quite different from treatment 2. The value obtained (77 m^2) was more similar to treatments 1 and 3, which were conducted on an adjacent site but in June and August, respectively. However, the shape of the area of attraction was different than treatment 2, indicating possible differences in the spatial current pattern for treatment 4.

The grouped recapture rates of the field tagged whelks from treatment 3 (33%) were significantly greater (test of percentages, $p < 0.05$; Sokal and Rohlf 1969) than those tagged in the laboratory (19%) and released at the same positions. The ratio of the two recapture rates indicated that the catchability of laboratory-tagged whelks was on average 58% lower than those tagged in the field. As expected from these results, treatment 8 yielded much higher recapture rates, resulting in a greater calculated effective area (276 m^2) than treatment 3, performed on approximately the same date and site, 1 yr earlier. These results suggested that the more stressful manipulations during the laboratory tagging decreased the catchability of the whelks, presumably by increasing the post-release recovery time, resulting in underestimates for the calculated effective areas of treatments 1 through 7.

Density Estimates

Density estimates, calculated from equation 1, were corrected for the stress factor ($\times 0.58$) for all treatments except

treatment 8 which was conducted using field-tagged animals. These density estimates, which ranged from 0.24 to 1.74 whelks·m^{-2} (Table 3), were comparable with the estimates from the intensive diving survey for the majority of the treatments (1, 3, 4, 7, and 8) in spite of the rather crude stress factor used to adjust for the reduced catchability of the laboratory-tagged animals.

Discussion

Our results support the hypothesis that the size and shape of the area of attraction and the size of the effective area of a baited whelk trap are influenced by physical and biotic factors. Although the treatments were not replicated, the results show that current speed and direction influence the size and shape of the area of attraction and that the effective area can vary by more than an order of magnitude depending on current conditions. Supplementary data suggested that the reproductive state of the whelks also affected the size of the effective area although this was not directly tested. Although certain treatment effects could not be explained, we have nonetheless presented a range of possible responses demonstrating clearly that the effective area is not a single, gear-specific value. These findings confirm our contention that density estimates calculated from catch per unit effort data may be considerably biased when assuming a constant effective area. Effective area estimates should be determined on a site- and season-specific basis if conditions known to affect the catchability of whelks, notably current strength and directionality and the reproductive state and feeding rates of the population, vary during the fishing season and/or over the fishing area.

Determination of the effective area and the subsequent estimation of population density using the tag–recapture method, described for this experiment, is inexpensive and can be performed relatively quickly. The effort is much less than for intensive diving surveys, while yielding similar results. Additional time could be saved by not releasing whelks at 50 m, as few whelks were captured from beyond 30 m after 1 d of fishing. The estimates obtained in this manner are direct and site specific, as opposed to the biased estimates resulting from a constant and/or modelized effective area, although replicates should be conducted to provide confidence limits to the estimates.

However, when conducting such experiments, care must be taken to ensure that the tagged population is representative of the natural population. Collecting whelks by divers near the treatment sites diminishes differences in catchability between the tagged and the natural populations by reducing (1) manipulation time and physiological stress, (2) behavioural differences due to differential reproductive or feeding activity, and (3) any possible biases introduced by collecting the experimental population with traps, i.e. some whelks may be prone to trapping or whelks may become less vulnerable, subsequent to being trapped. Although these biases were constant for treatments 1 through 7 and therefore should not affect the between treatment comparisons, they could affect the absolute effective area and density estimates.

Acknowledgements

We are indebted to Dr. Bob Miller for many helpful suggestions during the analyses of the data and to Dr. Bernard Sainte-Marie for reviewing an early draft of the manuscript. The capture, tagging, and release of the whelks for this experiment required the assistance of many people. We are particularly grateful for the aid of André Martel, Philippe Jalbert, Denis D'Amours, and Benoit Thomas. We are further indebted to Jorge Penereiro for taking responsibility for the execution of the 1984 treatment. Financial support was provided by grants from CRSAQ, CRSNG, and the Department of Fisheries and Oceans to J.H.H.

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Evaluation of Whole-Lake Nitrogen Fertilization for Controlling Blue-Green Algal Blooms in a Hypereutrophic Lake

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Lathrop, R. C. 1988. Evaluation of whole-lake nitrogen fertilization for controlling blue-green algal blooms in a hypereutrophic lake. Can. J. Fish. Aquat. Sci. 45: 2061–2075.

Indian Lake, a shallow hypereutrophic lake in southern Wisconsin, was treated with ammonium nitrate to test whether high dissolved inorganic nitrogen (DIN) concentrations or high total nitrogen to phosphorus (N:P) ratios could prevent the development of summer blue-green algal blooms (predominately *Microcystis aeruginosa*). The fertilizer was applied weekly from late April to early July 1981–82 in annual amounts of 14 and 23 g N/m^2, respectively. In 1981, a dense summer blue-green algal bloom developed, although both dissolved inorganic phosphorus (DIP) and algal P (particulate P/chlorophyll *a*) were lower than in summers without fertilizer treatments. In 1982, unusually clear water in May allowed aquatic macrophytes and associated filamentous algae to become very dense in June. The increase in water clarity and macrophytes was probably a response to a fishkill the previous winter. Later in the summer the macrophyte community disappeared and a blue-green algal bloom developed. After each fertilization in both treatment years, the NH_4^+ and NO_3^- decreased rapidly, suggesting nitrification/denitrification in the lake sediments. Whole-lake N fertilization did not prevent the summer blue-green algal blooms in Indian Lake. Low DIN apparently does not trigger the bloom development or cause the vernal nonblue-green algae to decline. Also, low total N:P ratios (<11 by weight) during the blooms occur partly because of high DIP and because of high algal P levels resulting from luxury P consumption. The Indian Lake data suggest that low total N:P ratios are only predictive of (or resultant from) blue-green algal blooms rather than causative.

Le lac Indian, dans le sud du Wisconsin (É.-U.), est une nappe d'eau peu profonde hypereutrophe; on y a appliqué du nitrate d'ammonium pour déterminer si une concentration élevée d'azote inorganique dissous (NID) ou un rapport d'azote à phosphore (N:P) totaux élevé peut empêcher l'apparition d'une fleur d'eau de cyanophycées (surtout *Microcystis aeruginosa*) en étæ. On a fait des applications d'engrais toutes les semaines de la fin d'avril jusqu'au début de juillet en 1981 et en 1982; la première année on a appliqué 14 g de N/m et la deuxième, 23 g de N/m^2. En 1981, il est apparu une fleur d'eau de cyanophycées dense durant l'été, même si la concentration de P inorganique dissous (PID) et la teneur en P algal (particules de P/chlorophylle *a*) étaient alors moindres que les étés où il n'y avait pas eu de traitement. En 1982, les eaux du lac étaient inhabituellement limpides en mai, si bien que les macrophytes aquatiques et les algues filamenteuses s'y associant sont devenus très abondants en juin. L'augmentation de la limpidité de l'eau et la multiplication des macrophytes étaient probablement une suite de la forte mortalité de poissons de l'hiver précédent. Plus tard durant l'été, la communauté de macrophytes est disparue et une fleur d'eau de cyanophycées est apparue. Les deux années, la concentration de NH_4^+ et de NO_3^- a baissé rapidement après le traitement, ce qui pourrait signifier qu'il y avait nitrification et dénitrification dans les sédiments lacustres. Le traitement global du lac par application d'engrais azoté n'a pas empêché l'apparition de fleurs d'eau de cyanophycées en été dans le lac Indian. Le fait que la concentration de NID soit basse ne semble ni déclencher la formation d'une fleur d'eau de cyanophycées ni entraîner la diminution des autres populations d'algues en période vernale. En outre, le rapport N:P totaux est faible (inférieur à 11, en poids) lorsqu'il y a des fleurs d'eau, en partie parce que la concentration de PID est élevée et parce que le P algal est important par suite d'une surabondance de consommation de P. D'après les données recueillies sur le lac Indian, le fait que le rapport N:P totaux soit faible n'est qu'un indice (ou le résultat) d'une fleur d'eau de cyanophycées et non pas une cause.

Received October 8, 1986
Accepted August 9, 1988
(J8967)

Reçu le 8 octobre 1986
Accepté le 9 août 1988

During the summer, shallow hypereutrophic lakes often have dense blue-green algal blooms coincident with low concentrations of inorganic nitrogen (N) and elevated concentrations of inorganic phosphorus (P) (cf. Barica 1974; Claesson and Ryding 1977; Coveney et al. 1977; Jones 1977; Olrik 1981). While P is generally the major plant nutrient stimulating lake eutrophication (Hutchinson 1957; Vollenweider 1968; Schindler 1977), N frequently is considered a growth-limiting nutrient in hypereutrophic as well as eutrophic lakes (Gerloff and Skoog 1957; Lund 1965; Claesson and Ryding 1977; Canfield 1983).

Although certain species of blue-green algae fix atmospheric N_2 to supplement their N requirements, many blue-green algal blooms are dominated by species that do not fix N_2 (Gerloff and Skogg 1957; Lund 1965; Horne 1978, 1979; Reckhow and Simpson 1980). Based on an analysis of survey data from north-

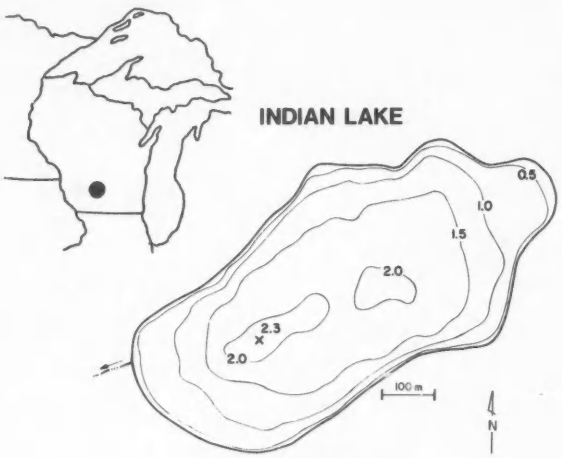

FIG. 1. Map of Indian Lake.

central and northeastern United States lakes of various trophic levels, the majority of lakes with blue-green algae were dominated by non-N_2-fixing species (Reckhow and Simpson 1980). Also, for lakes dominated by either blue-green algae or non-blue-green algae, the median surface concentrations of inorganic N were 0.2 and 0.8 mg/L, respectively, thus suggesting the adaptability of blue-green algae to lower N levels.

Total N:P ratios have been gaining widespread use as a management tool for indicating N-limiting conditions that favor blue-green algae (Canfield 1983; Forsberg et al. 1978; Schindler 1977; Smith 1983). Forsberg et al. (1978) reported that ratios <10 (by weight) are N limiting and >17 are P-limiting, and ratios 10–17 are either N limiting or P limiting or both. Smith (1983) suggested that total N:P ratios as high as 29 are needed to ensure the lack of blue-green algal blooms.

If the N:P ratio is a key factor regulating the development of blue-green algal blooms, then management programs that increase the ratio are desirable. To reduce algal biomass in lakes with low or moderate P levels, Schindler (1977) recommended increasing the N:P ratio by decreasing P supplies. In shallow hypereutrophic lakes, N loss mechanisms (e.g. nitrification/denitrification, ammonia volatization) coupled with efficient P recycling from the sediments leave few management options. While P recycling can be reduced by benthivorous fish control or by immobilization with aluminum or iron salts (Cooke et al. 1986), dredging may be the only effective management practice for long-term P control.

An alternative approach to increasing the N:P ratio in fertile lakes is to add N. If blue-green algal blooms result from the lack of inorganic N needed by other more desirable forms of algae, then artificially maintaining inorganic N above a certain threshold concentration should produce a difference in the dominant plankton.

Lake fertilization (both N and P) for fish farming has been practiced for centuries (Neess 1949), but the sole addition of inorganic N for the manipulation of algal blooms has only been tried experimentally in recent years. A number of studies have been made on oligotrophic or dystrophic systems (Sager 1963; Schindler and Fee 1974; Reuter et al. 1985). Only a few studies have reported the addition of inorganic N for the control of algal blooms in hypereutrophic systems. Barica et al. (1980) added ammonium nitrate to small enclosures in a lake and to a small pond. Large additions of inorganic N in the pond resulted in the disappearance of *Aphanizomenon flos-aquae* and a shift to small green algae and cryptomonads. The following summer with no inorganic N additions, a blue-green bloom similar to pretreatment conditions returned. In the enclosure experiments, a shift from the N_2-fixing *Aphanizomenon* to the nonfixing *Microcystis aeruginosa* occurred. Brownlee and Murphy (1983) reported the same shift in similar enclosure experiments. Leonardson and Ripl (1980) reported that NO_3^- caused the collapse of *Aphanizomenon* blooms and the subsequent replacement by sparse communities of cryptomonads and green algae.

The principle objective of this research project was to evaluate the whole-lake addition of ammonium nitrate fertilizer as a possible management tool for the reduction of blue-green algal blooms in fertile lakes. The results of the inorganic N fertilization, the effect on N and P levels in the lake, and the impact on the phytoplankton are summarized in this paper.

Study Area

Indian Lake is a shallow (2.3 m maximum depth, 1.4 m mean depth), hardwater, 27-ha kettle lake (Fig. 1) located on the edge of the terminal moraine and the "driftless area" (unglaciated) northwest of Madison, Wisconsin. The lake is surrounded by county parkland. Its small watershed is a mixture of woodland and farmland. Indian Lake has no stream inputs; the lake level is maintained by groundwater and precipitation. Water leaves the lake via an intermittent outlet only when lake levels are high.

Indian Lake has a history of severe summer blue-green algal blooms (Wisconsin Department of Natural Resources, unpubl. data) making the lake one of the most eutrophic in Wisconsin (Lillie and Mason 1983). The lake contains mostly nongame fish: bullheads (*Ictaluras melas*), green sunfish (*Lepomis cyanellus*), and fathead and bluntnose minnows (*Pimephales promelas* and *P. notatus*). The bullheads, along with wind action, rile the shallow lake sediments, increasing water turbidity and promoting nutrient recycling. Aquatic macrophyte growth has been sparse because of shading from the algal blooms. Periodic winter fishkills and occasional summer fishkills from oxygen depletion have occurred. Largemouth bass (*Micropterus salmoides*) have been stocked (last in 1979), but they are not numerous due to poor reproduction and the fishkills.

Methods

Limnological sampling on Indian Lake began regularly in May 1980 and continued through 1983. Sampling was done at the center of the lake; water samples were collected just below the surface with a 2.2-L Kemmerer. Tests conducted were water temperature, Secchi disc, dissolved oxygen (DO), pH, total alkalinity, total (TP) and dissolved inorganic phosphorus (DIP), total organic nitrogen (TON), and ammonium (NH_4^+) and nitrite/nitrate (NO_3^-) nitrogen summed as dissolved inorganic nitrogen (DIN). Dissolved organic nitrogen (DON) was analyzed in 1982–83. (All nutrient concentrations are reported as milligrams of element per litre.) The samples for TP, TON, NH_4^+, and NO_3^- were preserved with sulfuric acid beginning in May 1981, but all samples were chilled and then analyzed within 24 h following EPA (1979) procedures at the State Laboratory of Hygiene (SLOH) in Madison, Wisconsin. The dissolved nutrients were determined on samples after filtration through 0.45-μm membrane filters. DO (modified Winkler),

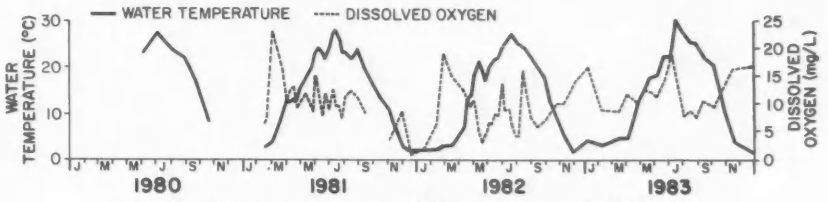

FIG. 2. Water temperature and DO concentrations, 1980–83.

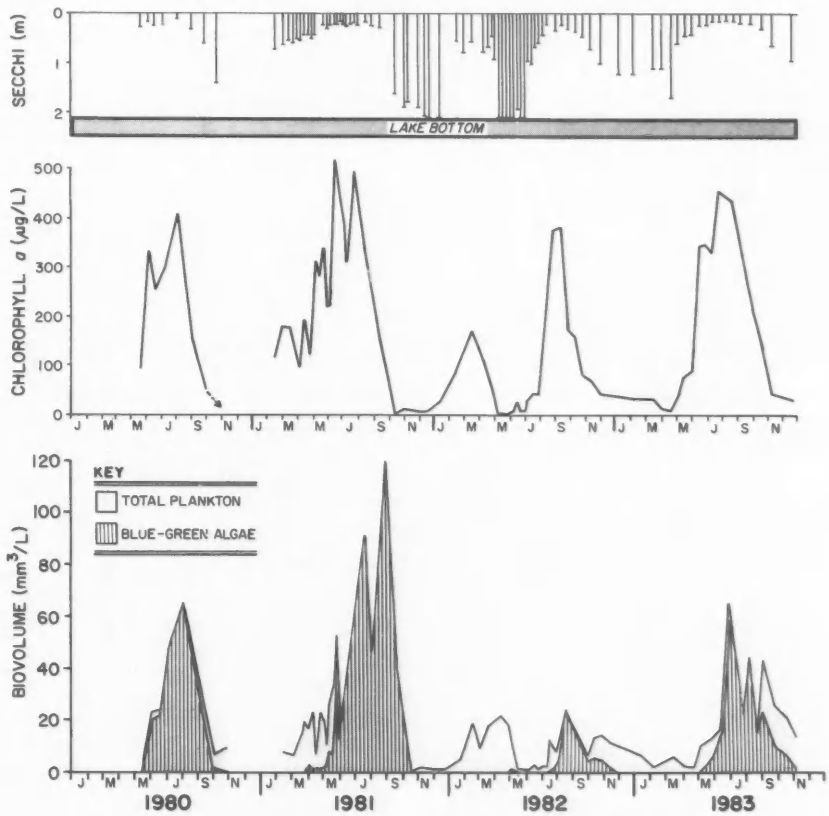

FIG. 3. Secchi transparencies, Chl *a* concentrations, and algal biovolumes, 1980–83.

pH, and alkalinity (pH end-point 4.5) were determined at the field station headquarters within 2 h of sampling following APHA (1976). The pH and alkalinity also were conducted at the SLOH. Unionized ammonia (NH_3) was estimated using NH_4^+, pH, and water temperature data (Thurston et al. 1979).

Samples for chlorophyll *a* (Chl *a*) and phytoplankton were collected from a 0–1 m composite. Chl *a* was determined after filtration through glass fiber filters and extraction in 90% acetone, using the Trichromatic technique (EPA 1979). The phytoplankton samples were preserved with Lugol's solution and later identified and volumetrically enumerated using the Utermohl technique.

Macrophyte samples (excluding roots) were collected during the first week of July 1981–83 and dried (105°C) for later analyses. P and N were determined at the University of Wisconsin-Madison Soil and Plant Analysis Laboratory by inductively coupled plasma emission spectrometry and the Kjeldahl technique, respectively.

Fertilizer Application

Indian Lake was fertilized with commercial-grade ammonium nitrate fertilizer (34–0–0) weekly from late April through early July of both 1981 and 1982. The fertilizer was applied to the lake in multiple batches of ~100 kg of dry pellets dissolved in 380 L of lake water pumped into a tank on a boat. The fertilizer solution was then pumped through a hose to the motor prop wash while the boat systematically traversed the lake. Each fertilizer treatment averaged 1090 kg (range 450–1820 kg) and 1650 kg (range 1270–1910 kg) for 1981 and 1982, respectively.

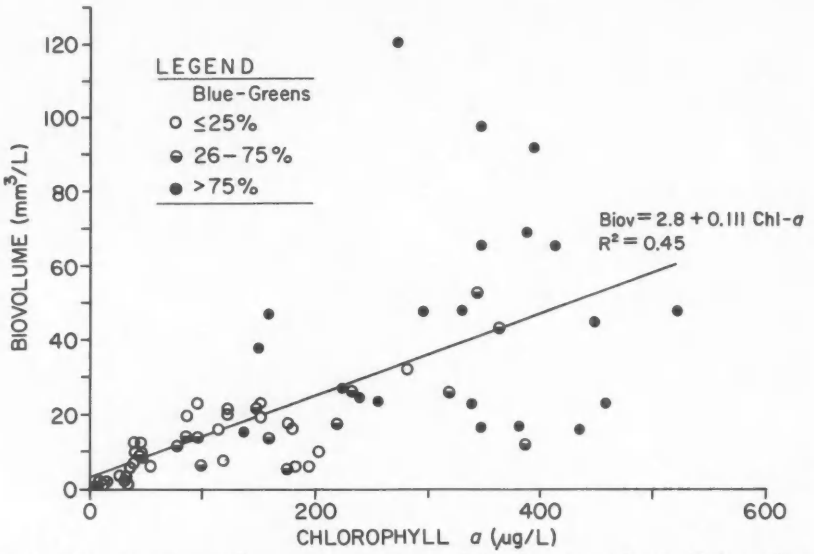

FIG. 4. Relationship between chlorophyll *a* and total phytoplankton biovolume (data stratified according to percent biovolume of blue-green algae).

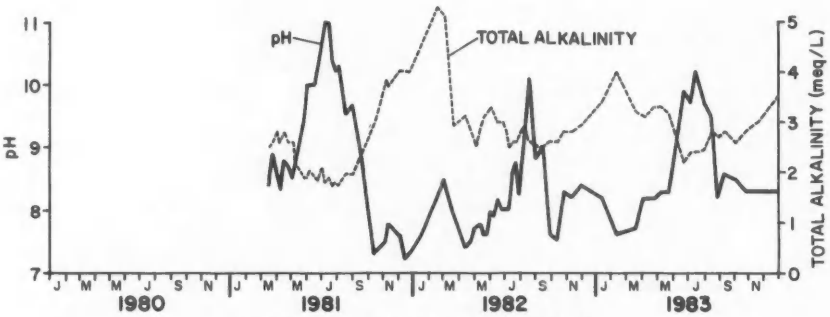

FIG. 5. Lake pH and total alkalinity, 1981–83.

The total amount applied was 10 900 (10 treatments) and 18 100 kg (11 treatments), corresponding to a N application of 14 and 23 g N/m² for 1981 and 1982. Less fertilizer was applied in 1981 than 1982, principally because of uncertainties about the amount needed by the algae, an underestimation of the depletion rate in the lake, and a concern for possible toxicity to fish from un-ionized ammonia. Fertilizer application also began a week earlier in 1982.

During the treatment phase, additional lake water samples were collected and analyzed for NH_4^+ and NO_3^- immediately before treatment and generally 1 and 4 d after treatment. Samples were also collected at three stations (east, center, west) in the lake 1 d after each treatment in 1981 to determine how evenly the fertilizer was applied and how rapidly the lake was mixing. Only the center station was sampled in 1982, because NH_4^+ and NO_3^- concentrations at the east and west stations usually differed by <10% of concentrations at the lake center.

Results

Water Temperature and DO

Indian Lake, because of its shallowness, responded rapidly to seasonal air temperatures (Fig. 2). Surface water temperatures reached at least 27°C during July 1980–83. Ice covered the lake by late November until late February – early March during the study years.

The surface water generally was well oxygenated on the days sampled except for a few occasions (Fig. 2). In early November 1981 before the lake surface froze, DO concentrations were quite low (3.7 mg/L) and undersaturated (33%) during a clear-water period. Soon after the ice formed, DO dropped to <1 mg/L causing a fishkill. Relatively low DO (3–5 mg/L) also was recorded in late May during another clear-water period and in late July 1982. Supersaturation frequently occurred during the summer blue-green algal blooms.

Secchi Disc, Chlorophyll *a*, and Phytoplankton

Bloom development, 1980–83

Indian Lake had a dense blue-green algal bloom beginning in late May and extending through the pretreatment summer of 1980 (Fig. 3). Secchi transparencies were <0.3 m and Chl *a* was 250–400 µg/L. In June the bloom was predominantly *Anabaena flos-aquae*, but from July through early September,

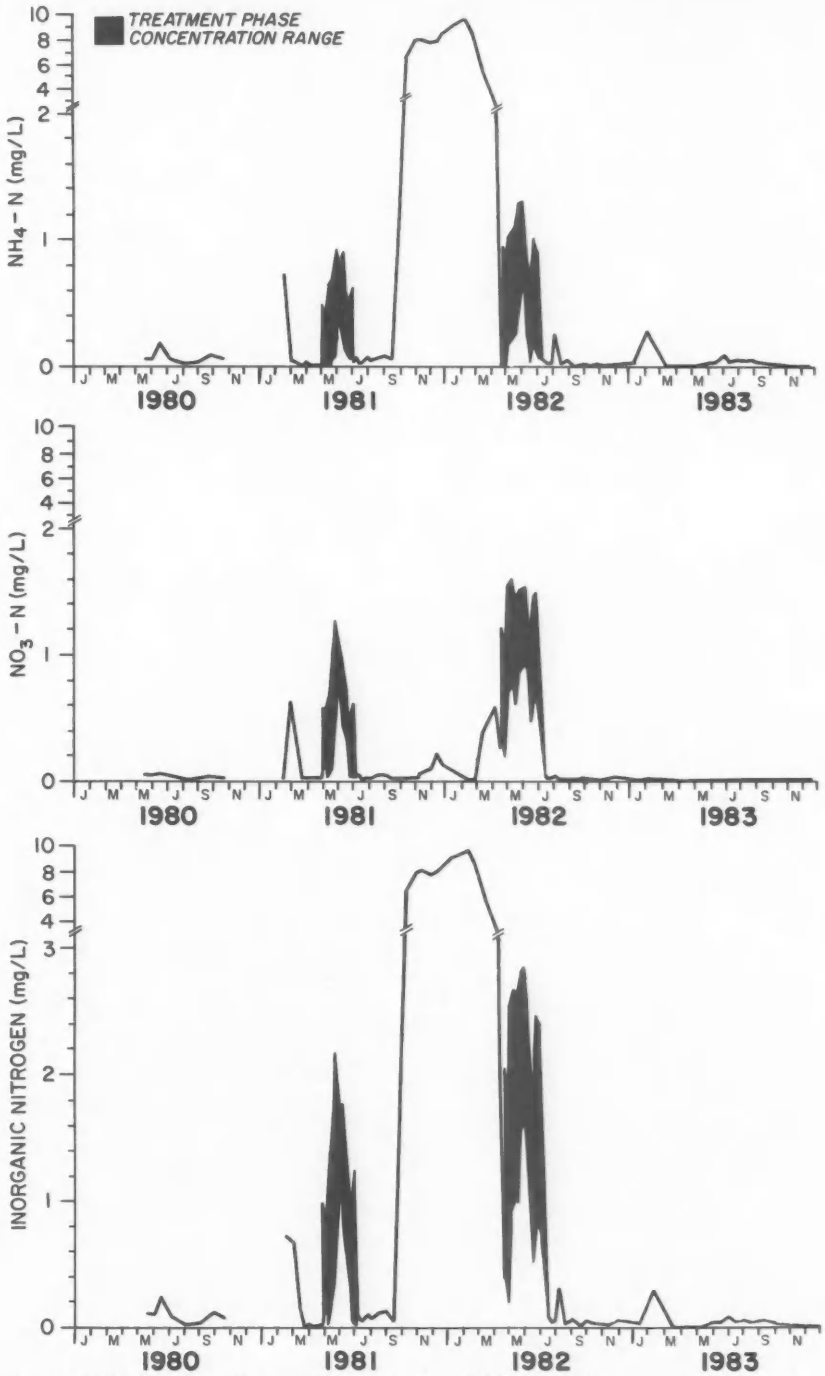

FIG. 6. NH_4-N, NO_3-N, and inorganic N concentrations, 1980–83, including concentration ranges during N fertilization in 1981–82.

Microcystis aeruginosa dominated; *Aphanacapsa pulchra* and *Chroococcus dispersus* were also present. Water clarity improved (Secchi 1.4 m) by late October 1980; the phytoplankton were mostly cryptomonads and diatoms.

In mid-February 1981, an algal bloom of chlorophytes and chrysophytes occurred under the ice and continued into the spring with Secchi transparencies of 0.4–0.6 m and Chl *a* 90–200 μg/L. In late May 1981, almost 1 mo after the weekly

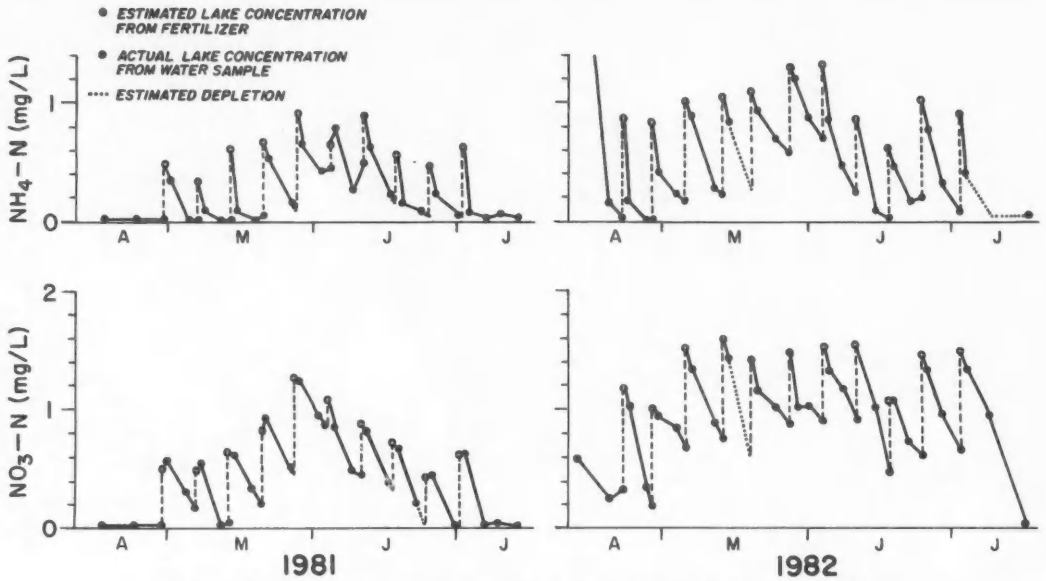

FIG. 7. NH_4-N and NO_3-N concentrations during N fertilization from April to July, 1981–82.

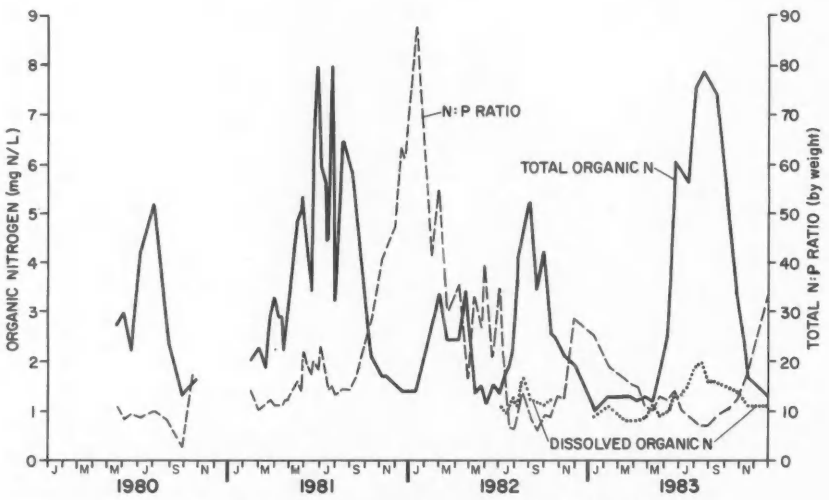

FIG. 8. TON and DON concentrations and total N:P ratios, 1980–83.

fertilizer treatments began, a dense blue-green algal bloom developed that lasted through September. During the summer, Secchi transparencies were ~0.2 m and Chl *a* was 250–500 μg/L. In late May, the blue-green bloom was mostly *Microcystis aeruginosa*. In June, *Chroococcus dispersus*, *Anabaena scheremetievi*, *Anabaena spiroides*, and *Aphanocapsa pulchra* were the principal species. From early July after N fertilization ceased, *Microcystis* comprised almost 100% of the plankton biovolume through mid-August. *Aphanocapsa*, *Microcystis*, and *Oscillatoria agardhii* were important in September.

During the fall of 1981, the lake water progressively cleared (Secchi ~2.0 m and Chl *a* ≤12 μg/L) until a cryptomonad bloom developed under the ice in February 1982. The bloom continued after ice-out through March and April (Secchi 0.3–0.8 m and Chl *a* 100–200 μg/L). By mid-May (after the fertilization had begun), the Secchi disc was easily seen on the lake bottom (2.3 m) and Chl *a* was low (0–10 μg/L). By the second week in June, *Volvox globator*, a large colonial species found in waters rich in nitrogen (Prescott 1962), became common although the total phytoplankton biovolume was sparse. During the clear-water periods in the fall of 1981 and spring of 1982, large populations of *Daphnia pulex* probably accounted for the low algal biovolumes.

The phytoplankton began to increase shortly after the last fertilizer treatment in early July 1982. In mid-July the diatom *Melosira granulata* was the major species. By the end of July,

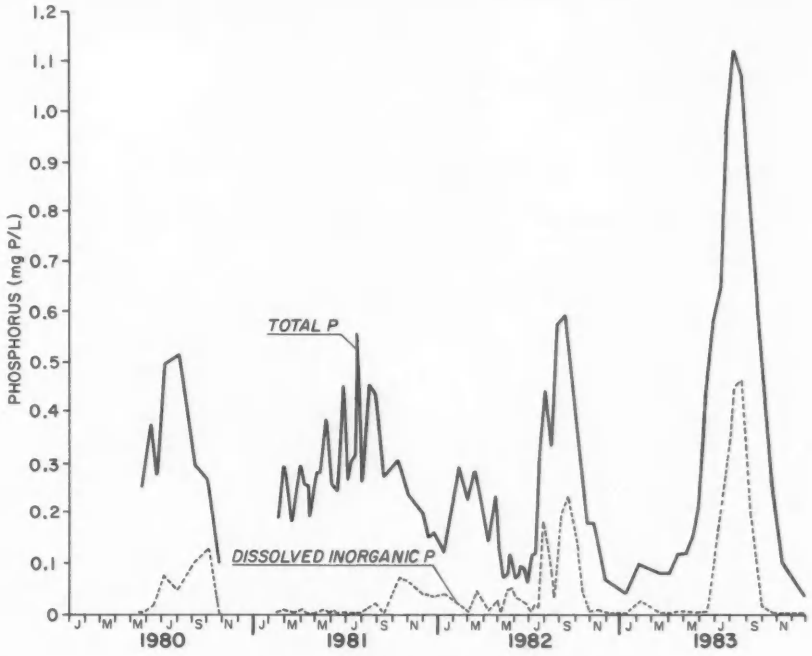

FIG. 9. TP and DIP concentrations, 1980–83.

the blue-green algae *Anabaena circinalis* and *Anabaena spiroides* codominated with *Melosira*. Both blue-greens dominated the plankton throughout August. In September, *Anabaena spiroides*, *Aphanocapsa rivularis*, *Chroococcus dispersus*, and *Microcystis aeruginosa* occurred. During the late summer blue-green algal bloom, Secchi transparencies and Chl *a* were similar to data from other summer blooms, but biovolumes were lower.

Blue-green algal abundance decreased after September 1982, although a diverse nonblue-green algal community persisted and water clarity improved only slightly in October and November (Secchi 0.5–0.7 m, Chl *a* 75–86 μg/L). Water clarity further increased after the lake surface froze in late November (Secchi 1.0–1.2 m, Chl *a*, 34–46 μg/L) and remained relatively constant until April 1983, 1 mo after ice-out. No major phytoplankton group or species dominated.

In late April 1983, water clarity increased briefly (Secchi 1.7 m) before a moderate algal bloom occurred from mid-May to mid-June (Secchi ~0.4 m, Chl *a* 77–95 μg/L). Although the blue-green algae started to develop in mid-May, nonblue-green algae comprised more than half of the plankton biovolume until mid-June. By the third week in June 1983, a dense blue-green bloom developed and remained into September. Secchi transparencies were 0.15–0.22 m and Chl *a* was 347–449 μg/L. *Anabaena circinalis* was the most dominant species in late June. In the first half of July, *Chroococcus limneticus* and *Microcystis aeruginosa* codominated, with *Chroococcus* becoming the dominant species by mid-August. In early September 1983, nonblue-green algae began to appear in the plankton, but water clarity remained poor through October (Secchi ≤0.3 m, Chl *a* ≥147 μg/L). By early November, the blue-green algae had been replaced by a diverse community of nonblue-greens.

Chl a/biovolume relationships

Based on the entire 1980–83 data set, Chl *a* and phytoplankton biovolume were linearly related ($R^2 = 0.45$), but variability increased for larger concentrations of Chl *a* (Fig. 4). The dense algal blooms (Chl *a* >200 μg/L) were predominately blue-green algae. When Chl *a* was <50 μg/L, blue-green algae were generally ≤25% of the total biovolume.

Macrophytes

Aquatic macrophytes grew only in very sparse stands on each end of the lake during the summers of 1980 and 1981. The dominant species were the narrow-leaved pondweeds *Potamogeton pectinatus* and *Potamogeton berchtoldii*. During the spring of 1982, the macrophyte community proliferated when the lake water became clear. By early June, *Potamogeton pectinatus* and *Potamogeton berchtoldii* were growing in dense stands around the lake from the shoreline to water depths of 1.3–1.7 m, eventually covering 44% of the lake area. By the third week in June, the filamentous alga *Hydrodictyon* sp. started growing as a thick yellow mat on top of the macrophytes near the shore. Within 2 wk (July 2) this mat had spread, covering almost the entire macrophyte community. One week later the *Hydrodictyon* mat had almost completely disintegrated. The macropytes also underwent rapid senescence. By late July, macrophytes were relegated to minor patches at both ends of the lake.

In mid-May 1983, dense macrophytes redeveloped. Although *Potamogeton pectinatus* and *Potamogeton berchtoldii* were still important species, *Potamogeton crispus*, which was only found in isolated patches in 1982, spread throughout the community in dense stands. However, the filamentous algal bloom did not

reappear. In late June, the macrophyte community underwent senescence as the blue-green algal bloom took over.

Macrophyte N and P concentrations were not greatly affected by the N fertilization, despite large differences in water column N and P. For 1981–83, N in percent dry weight was 2.1, 3.2, and 3.1 and P was 0.43, 0.38, and 0.50, respectively.

pH and Alkalinity

High midday pH values (9.5–11.0) occurred during the summers when the blue-green algal blooms were dense (Fig. 5). The pH was generally 8.0–8.8 during the fall, winter, and spring months when the less dense, nonblue-green algal blooms occurred. The pH was 7.2–7.9 during periods of clear water.

Alkalinity varied between 2 and 5 meq/L and averaged about 3 meq/L during the study period (Fig. 5). Alkalinity was lowest during the blue-green algal blooms and was highest during the clear-water periods.

Nitrogen

Inorganic nitrogen

NH_4^+ was generally low (<0.02–0.09 mg/L) and NO_3^- was often undetectable (<0.02 mg/L) during the algal blooms that occurred from late March through November, except during the periods of fertilizer treatment and under the ice in late winter (Fig. 6). In 1981, DIN was undetectable from the third week of March until the fertilizer was first applied on April 29. However, in October 1981 when the lake water became clear, NH_4^+ increased to unusually high levels (>6.5 mg/L) that remained through early March 1982. In 1982, NO_3^- was still present (0.34 mg/L) before the fertilizer application on April 22 because of the large reservoir of DIN remaining from the previous winter. NH_4^+ was not depleted until 1 wk prior to fertilization in 1982.

Lake concentrations of NH_4^+ and NO_3^- dropped rapidly after each fertilizer treatment; NH_4^+ generally decreased faster (Fig. 7). The NH_4^+ and NO_3^- depletion rates of the applied fertilizer were statistically similar between treatment years ($\alpha = 0.05$), averaging 240 and 86 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$ (340 and 120 mg $N \cdot m^{-2} \cdot d^{-1}$) for NH_4^+ and NO_3^-, respectively. However, the variability of the NH_4^+ depletion was high. The lowest loss rates of NH_4^+ (50–120 $\mu g \cdot L^{-1} \cdot d^{-1}$) coincided with the clear water period in May 1982. For comparison, the depletion rate of NH_4^+ during March and April 1982 averaged 150 $\mu g \cdot L^{-1} \cdot d^{-1}$ (210 mg $N \cdot m^{-2} \cdot d^{-1}$).

After the first three treatments in 1981, the NH_4^+ was depleted by the end of the week before the next treatment, while NO_3^- was depleted only after the second treatment (Fig. 7). In 1982, the NH_4^+ was depleted only by the end of the week after the first treatment in late April and briefly during mid-June. The NO_3^- was never depleted during the 1982 treatment phase.

NO_3^- was about 50% higher than NH_4^+ throughout the treatment phase of both years because of the more rapid NH_4^+ depletion and the resultant lower initial NH_4^+ concentration before each subsequent fertilization. Also, both NH_4^+ and NO_3^- were higher in 1982 than 1981 because greater amounts of fertilizer were applied. However, for both 1981 and 1982, relatively high DIN concentrations were maintained in Indian Lake by mid-May before the blue-green algal blooms typically developed (Fig. 6). DIN was generally >0.50 mg/L, and frequently >1.0 mg/L. Shortly after the treatments ended in early July, low DIN levels returned.

Organic nitrogen

During the summer blue-green algal blooms, TON was generally >4 mg/L. Maximum concentrations were measured at ~5 mg/L in 1980 and 1982 and 8 mg/L in 1981 and 1983 (Fig. 8). TON generally varied between 1.5 and 3.0 mg/L during the nonblue-green algal blooms in the spring and fall. During periods of clear water, TON was 1.0–1.5 mg/L. DON was much less variable than TON. Consequently, most of the variation in TON was in particulate organic nitrogen (PON), a function of phytoplankton standing crop.

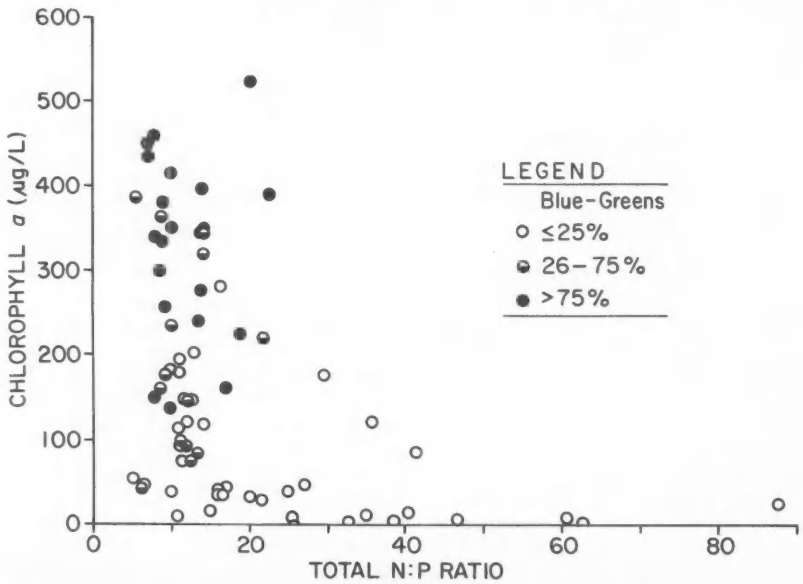

FIG. 10. Relationship of total N:P ratios and Chl *a* concentrations (data stratified according to percent biovolume of blue-green algae).

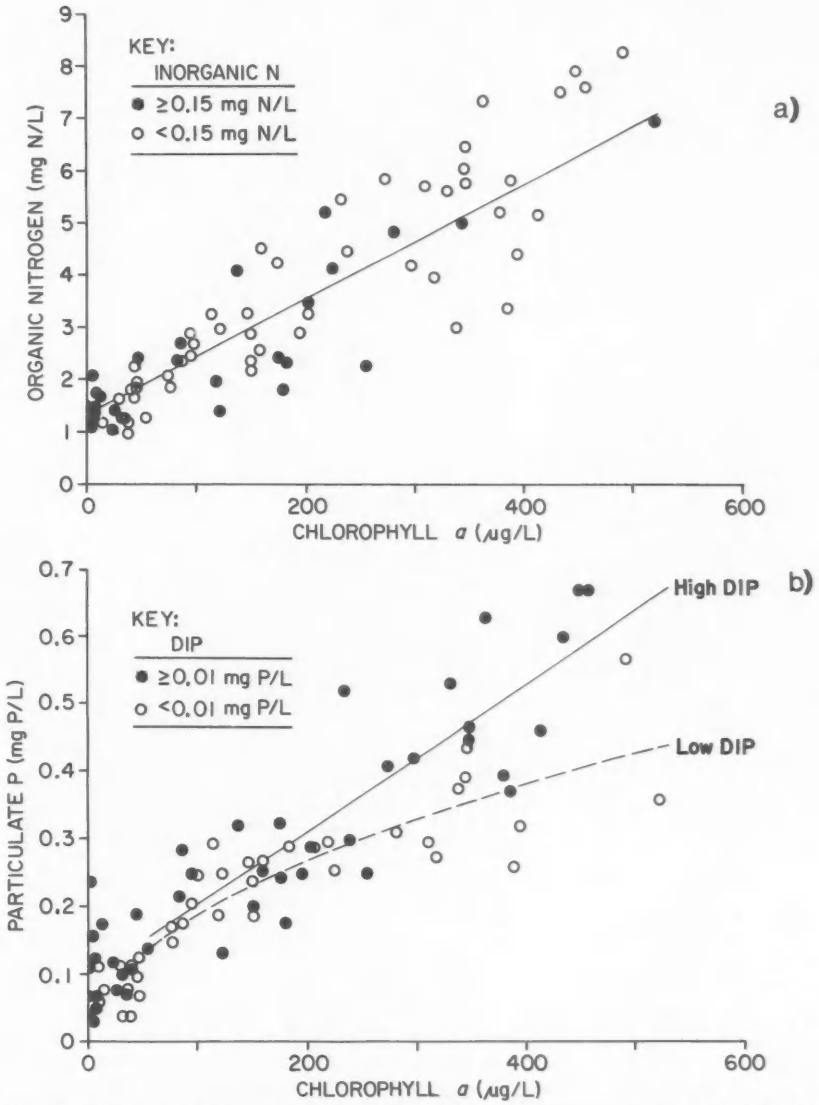

FIG. 11. Relationship of Chl *a* to (a) TON and (b) PP (P data stratified according to low or high DIP; N data stratified according to low or high DIN). TON = 1.37 + 0.011 Chl *a*, R^2 = 0.70; PP = 0.09 + 0.0011 Chl *a*, R^2 = 0.84 (high DIP); PP = −0.01 + 0.0194 $\sqrt{\text{Chl } a}$, R^2 = 0.79 (low DIP).

Un-ionized ammonia

Un-ionized NH_3 in Indian Lake was generally <0.02 mg/L during the spring and fall months and 0.03–0.09 mg/L during the summer blue-green algal blooms when the daytime pH was >9.0. However, un-ionized NH_3 was also high during the blue-green algal bloom in 1981 when NH_4^+ concentrations were high because of the fertilizer treatments. Because the daytime pH was frequently >10.0, NH_3 was ≥80% of the NH_4^+ concentration. NH_4^+ averaged 0.66 mg/L immediately after the four fertilizer treatments in June. Average NH_4^+ levels had dropped to only 0.30 mg/L by 4 d after treatment for those weeks and was still >0.16 mg/L prior to each fertilization until the last treatment in June. Consequently, the un-ionized NH_3 was greater than 10 times the EPA (1976) criterion of 0.02 during the day for at least the first 3 wk of June. Nighttime pH (not sampled) was probably lower due to algal respiration, such that these elevated NH_3 levels were not continuous. NH_3 during the large increase of NH_4^+ in the fall of 1981 was only 0.01–0.09 mg/L because the pH was <8.0 and water temperatures were cool. NH_3 increased to 0.28 mg/L under the ice in March 1982 because the pH increased to 8.5 and NH_4^+ levels remained high.

An important unanswered question is whether the high levels of NH_3 in June 1981 were sufficiently toxic to cause a fish population reduction that allowed *Daphnia pulex* populations to be high during the clear-water periods in the fall of 1981 and the spring of 1982. No fishkill was observed during the 1981

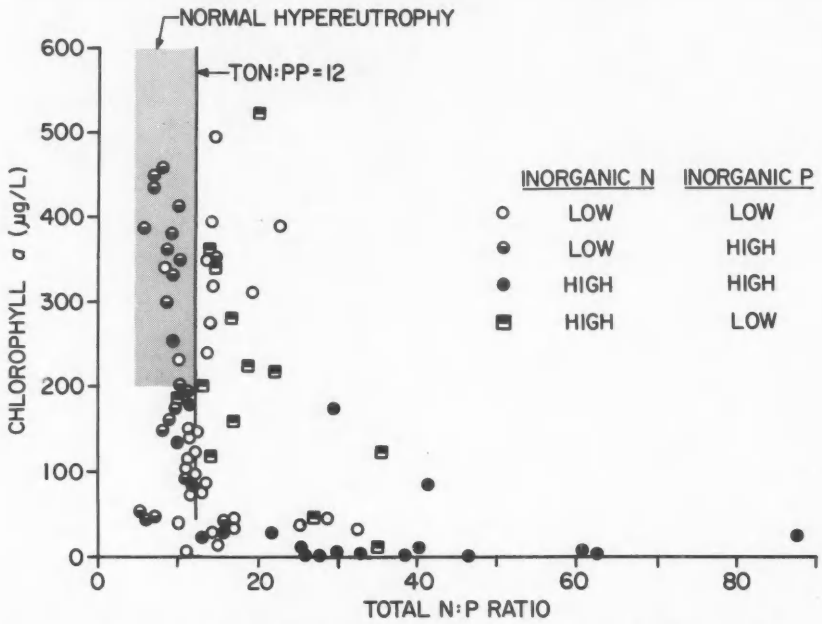

FIG. 12. Relationship of total N:P ratios and Chl *a* concentrations (data stratified according to low or high DIN and DIP, as in Fig. 11).

open water period. Fish were found during a fall survey, although only medium-sized green sunfish were numerous. Minnows were not abundant. Acute toxicity levels (96-h LC50) for largemouth bass, fathead minnows, and juvenile green sunfish are all ≥0.70 mg/L (McCormick et al. 1984; Roseboom and Rickey 1977; Thurston et al. 1983), which is higher than the 4-d average NH_3 concentration after each June fertilizer treatment.

Phosphorus

TP was generally >0.25 mg/L throughout the summer blue-green algal blooms (Fig. 9). Concentrations peaked at 0.50–0.60 mg/L in the summers of 1980–82 and at ~1.10 mg/L in 1983. TP was 0.10–0.25 mg/L during the spring and fall nonblue-green algal blooms. TP was <0.10 mg/L when the water was relatively clear.

DIP normally was elevated during the blue-green algal blooms; concentrations were >0.06 mg/L and often >0.10 mg/L (Fig. 9). During the summer of 1983, DIP peaked at 0.46 mg/L. However, DIP was generally ≤0.004 mg/L during the blue-green algal bloom in 1981 (treatment year). DIP was usually <0.004 mg/L and was never recorded above 0.009 mg/L during the spring and fall nonblue-green algal blooms. DIP varied between 0.020 and 0.070 mg/L during clear-water periods.

Total N:P Ratios and Nutrient Limitation

Total N:P ratios were <11 (by weight) during the normal summer blue-green algal blooms (Fig. 8). Ratios were typically 11–20 in the spring and fall when the nonblue-green algal blooms occurred. During the blue-green algal bloom in the summer of 1981, N:P ratios increased to an average of 19. After the fertilizer treatments had ended in late July and August, N:P ratios dropped to ~14. N:P ratios were high from the fall of 1981 until the early spring of 1982 because of the high NH_4^+ concentrations. N:P ratios averaged 28 throughout the 1982 treatment period when the water was clear, but ratios later dropped to <11 as the blue-green algal bloom developed.

An analysis of Chl *a* versus N:P ratios indicated that when ratios were ≥25, blue-green algal blooms did not occur (Fig. 10). N:P ratios were highly variable for samples having Chl *a* <50 µg/L; phytoplankton were mostly nonblue-green species. Generally, samples having Chl *a* from 100 to 200 µg/L were not dominated by blue-green algae but had relatively low N:P ratios (~12).

Of particular interest was whether nutrient limitation occurred during both blue-green and nonblue-green algal blooms when Chl *a* was >50 µg/L. N and P limitation were estimated by the presence of low concentrations of DIN (<0.15 mg/L) and DIP (<0.01 mg/L), respectively. TON, particulate phosphorus (PP, estimated by TP minus DIP), and Chl *a* data were classified by (1) low DIN, low DIP; (2) low DIN, high DIP; (3) high DIN, low DIP; (4) high DIN, high DIP. Figure 11a shows TON plotted against Chl *a* for both low and high DIN. No major differences between the two DIN classifications were observed. Apparently, inorganic N fertilization in 1981 did not cause an increase in organic N in the blue-green algae when compared with organic N levels in algae during the normal summer blooms when DIN was low. TON/Chl *a* remained relatively constant.

Figure 11b shows PP plotted against Chl *a* for both low DIP and high DIP. The PP/Chl *a* relationship for each DIP classification was similar for Chl *a* levels between 50 and 200 µg/L (low proportion of blue-green algae). However, for Chl *a* levels >200 µg/L (large proportion of blue-green algae), PP concen-

trations were greater when DIP was high during nontreatment summers than when DIP was low during the 1981 summer of N fertilization. Regression analyses indicated a good linear fit for the high DIP data and a curvilinear fit for the P-limiting data. Consequently, when DIP was in excess, PP/Chl *a* was relatively constant for all levels of algal standing crop. When DIP was low, PP/Chl *a* decreased as Chl *a* increased above 200 μg/L.

The levels of DIN and DIP greatly influenced total N:P ratios for various degrees of algal blooms (Fig. 12). During normal summers exhibiting hypereutrophic conditions, DIN was low but DIP was high, allowing luxury uptake of P by the blue-green algae. The result was relatively high TP that caused N:P ratios to be lower than the average TON:PP ratio of 12 found for all water samples analyzed. (The linear regression equation of TON versus PP had a slope of 12 and an R^2 of 0.84.) Total N:P ratios were higher during the blue-green algal bloom in 1981 (fertilized) because DIP was low, which also resulted in a concomitant lower level of algal PP. TN increased only slightly because of the N fertilizer, but TP was significantly lower. Ratios remained higher than normal in July and August of 1981 after the DIN had been depleted because of the continuing low levels of DIP and PP.

Many algal blooms not dominated by blue-green algae (Chl *a* 50–200 μg/L) had both low DIN and low DIP (Fig. 12). Total ratios were around 12, the average TON:PP ratio. Total ratios were higher for a few samples in this Chl *a* range because DIN was high, while DIP was low. For samples with Chl *a* <50 μg/L, total N:P ratios were highly variable because of elevated DIN and DIP; the higher ratios were caused by high concentrations of NH_4^+. DON also constituted a much larger portion of TN because of the lower standing crop of algae.

Discussion

During the summer blue-green algal blooms, Indian Lake had water quality conditions typical of hypereutrophic lakes: TP, DIP, TON, Chl *a*, and pH were high and DIN, total N:P ratios, and Secchi disc transparencies were low. Summer blue-green algae in Indian Lake were mostly non-N_2-fixers. *Microcystis aeruginosa*, a major bloom species in southern Wisconsin lakes (Gerloff and Skogg 1957; Wisconsin Department of Natural Resources, unpubl. data), was the predominant species during July and August. *Anabaena* spp. were the only important N_2-fixers, found mostly in June. This relative lack of N_2-fixing blue-green algae in Indian Lake contrasted with the N_2-fixing *Aphanizomenon flos-aquae* blooms in the hypereutrophic lakes where algal responses to N fertilization were reported (Barica et al. 1980; Brownlee and Murphy 1983; Leonardson and Ripl 1980).

Indian Lake did not respond as expected to the N fertilizer treatments in 1981 and 1982. In 1981, high levels of DIN did not prevent the development of a summer blue-green algal bloom, although normally high summer DIP levels were reduced to ≤0.004 mg/L. Low DIP may have restricted luxury P consumption by the blue-green algae, resulting in lower PP/Chl *a* levels. The cause of this low summer DIP may have been linked to NO_3^- oxidation of iron (Fe) in the sediments (Andersen 1982; Bostrom et al. 1982) given the high N loadings. However, other mechanisms related to bacterial processes may have caused the low DIP (B. Bostrom, Institute of Limnology, Uppsala, Sweden, pers. comm.) because DIP was higher during the fertilizer treatments in 1982. Southern Wisconsin lakes also have much of their Fe tied up as insoluble FeS and thus have a reduced capacity for restricting P mobility from oxidized sediments (Holdren 1977; Stauffer 1987).

The absence of a blue-green algal bloom in early summer 1982 seems more the result of fish and zooplankton population changes associated with the fishkill the previous winter. Fewer benthivorous fish would have resulted in less recycling of nutrients from the lake sediments and fewer planktivorous fish would have reduced the predation pressure on the herbivorous zooplankton community. A large *Daphnia* population in the spring of 1982 also may have helped prevent the early onset of the blue-green algal bloom. This result suggests the role that biomanipulation may have in affecting the symptoms of eutrophication.

The clear water in mid-May 1982 allowed aquatic macrophytes (*Potamogeton* spp.) to spread rapidly. This macrophyte proliferation was typical of shallow fertile lakes after a fishkill (Wisconsin Department of Natural Resources, unpubl. data), which Indian Lake experienced the previous winter. Thus, the macrophyte growth was probably not in response to the N fertilization because growth was sparse in 1981. The blue-green algal blooms that followed the mid-summer senescence of the *Potamogeton* macrophyte community in 1982 and 1983 also have occurred in more recent years in Indian Lake and have been observed in another nearby impoundment (Engel 1985).

Plant concentrations of N and P did not reflect different water concentrations of DIN and DIP in 1981–83. Howard-Williams (1981) reported an increase of N and P in *Potamogeton pectinatus* after the water was fertilized with both nutrients, but his unfertilized plant concentrations were much lower than in Indian Lake. Macrophyte N and P levels in Indian Lake were similar to averages from other studies (Hutchinson 1975) and were well above Gerloff's (1973) minimum tissue concentrations needed for growth. Thus, the macrophytes probably obtained their nutrients for growth from the sediments, as has been found for most rooted submerged macrophytes (Wetzel 1983).

The filamentous algal mat (*Hydrodictyon* sp.) that grew on top of the macrophytes from mid-June to mid-July 1982 may have been caused by the fertilization. In other studies, epiphytic algal blooms (*Cladophora* and *Oedogonium*) have developed when the water was heavily enriched with N and P (Howard-Williams 1981; Moss 1976). Filamentous algae did not reappear in Indian Lake in 1983 when the macrophytes were dense and DIN and DIP were low.

Nitrogen Cycling

Frequent fertilizer treatments were required to maintain high DIN in Indian Lake. This rapid depletion of fertilizer N has been reported in other studies (Neess 1949; Bouldin et al. 1974; Barica et al. 1980; Jana and Patel 1985). Mechanisms that may explain the N loss are (1) biological assimilation, (2) NH_3 volatilization, and (3) nitrification/denitrification (Brezonik 1972; Goering 1972; Keeney 1973; Wetzel 1983). Algae assimilate NH_4^+ in preference to NO_3^- (Dugdale and Dugdale 1965; Liao and Lean 1978; Kappers 1980; McCarthy 1980; Takahashi and Saijo 1981). NH_3 volatilization can account for significant losses of NH_4^+ when the pH is >9.0 (Bouldin et al. 1974; Murphy

and Brownlee 1981; Weiler 1979). Nitrification of NH_4^+ to NO_3^- occurs at the sediment–water interface where nitrifying bacteria are prevalent (Brezonik 1972; Chen et al. 1972a). Denitrification of NO_3^- to N_2 occurs under anaerobic conditions in the sediments where organic C and denitrifying bacteria (facultative anaerobes) are abundant (Neess 1949; Chen et al. 1972b; Keeney 1973; Tiren 1977; Jana and Patel 1985; Kaspar 1985). In fertile lakes, denitrification is considered one of the most important mechanisms for N loss.

While both algal assimilation and NH_3 volatilization partly explain the higher average loss rate of NH_4^+ (240 $\mu g \cdot L^{-1} \cdot d^{-1}$) versus NO_3^- (86 $\mu g\ N \cdot L^{-1} \cdot d^{-1}$) in Indian Lake, NO_3^- generated by nitrification may have contriubted to the slower depletion of the fertilizer NO_3^-. In 1982, NH_4^+ losses by either algal assimilation or NH_3 volatilization should have been less important than in 1981 because the phytoplankton standing crop and the pH were much lower. However, NH_4^+ depletion rates between the two years were not statistically different. Consequently, nitrification/denitrification may have been the major pathway for the loss of the fertilizer N in Indian Lake.

Rapid DIN loss suggests that various supply mechanisms must have been operating to provide adequate DIN for phytoplankton growth. Turnover rates must have been high during both the blue-green and nonblue-green algal blooms because DIN concentrations were low. High DIN probably cannot be maintained because of nitrification/denitrification.

DIN may have been added to the water column through ammonification: the regeneration of NH_4^+ from (1) bacterial decomposition of nitrogenous organic compounds, (2) direct autolysis of algal cells, or (3) zooplankton excretion (Brezonik 1972; Coveney et al. 1977). In hypereutrophic lakes, large reserves of organic N in the sediments (Keeney et al. 1970; Isirimah et al. 1976) and dense algal blooms suggest that the first two forms of ammonification may have been important in Indian Lake. Bacterial ammonification probably caused the large increase in NH_4^+ during the fall of 1981 and the lower DO prior to the lake freezing over. Nitrification of the NH_4^+ to NO_3^- with subsequent denitrification also may have lowered DO levels.

Although zooplankton have been identified as an important contributor of NH_4^+ in many lakes (Lean et al. 1982; Lehman 1980), their normally low numbers in Indian Lake suggest they were unimportant. The use of published NH_4^+ excretion rates (Jacobsen and Comita 1976; Lehman 1980) also indicates that even when *Daphnia pulex* were abundant (50/L) in the fall of 1981, only 0.28–0.46 mg N/L would have been produced, while NH_4^+ increased to >6.5 mg/L.

N_2-fixation by blue-green algae can add N to lakes, but it normally contributes only a minor proportion of a lake's N needs (Horne 1978). In Indian Lake, N_2-fixation by blue-green algae probably is only of limited importance because non-N_2-fixing *Microcystis aeruginosa* dominated in July and August. Some N_2-fixation may have occurred in June when N_2-fixing *Anabaena* spp. were present. The role of N_2-fixing bacteria in Indian Lake is unknown, although it has been considered a major source of N in other fertile lakes and ponds (Neess 1949).

Importance of N and P during Algal Blooms

The original hypothesis that blue-green algal blooms in Indian Lake are caused by low DIN was not substantiated. The onset of the summer blue-green algal blooms was not triggered by low DIN levels, which occurred much earlier in the spring before the summer blooms developed. The predominance of non-N_2-fixing *Microcystis aeruginosa* during the summer suggests that adequate supplies of N were available even though DIN concentrations were low. *Microcystis* also has a competitive advantage because of a low N requirement (Horne 1978).

The response of the blue-green algae to the fertilizer also suggests that DIN is not the growth-limiting nutrient in Indian Lake. High levels of fertilization in 1981 did not prevent the development of the summer blue-green algal bloom or cause a major difference in the succession of nonblue-green to blue-green algae when compared with the pretreatment summer of 1980. The success of N fertilization for eliminating *Aphanizomenon flos-aquae* blooms reported in other studies may have been because the systems were N limited, with N_2-fixation supplying the N needed for algal growth.

The occurrence of nonblue-green algal blooms during the spring and fall of many years when DIN and DIP were low is of interest. Because growth is not generally limited by both nutrients simultaneously (Kilham and Kilham 1984; Rhee 1978), P alone may have been limiting growth during the spring and fall blooms. Hence, low DIN levels may not indicate N limitation at all. Similar findings were reported by Lean et al. (1982) for a number of lakes having different trophic levels.

P probably was not growth limiting during the normal summer blue-green algal blooms when DIP levels were elevated. Brownlee and Murphy (1983) and Prepas (1983) have reported that high DIP indicates slow P turnover rates and no P limitation to algae. In Indian Lake, the high DIP apparently also allowed luxury consumption of P by the blue-green algae. When DIP was low in the summer of 1981 (fertilized), the blue-green algae had lower cellular P levels.

One factor that may have been growth limiting to the blue-green algal blooms in Indian Lake was light. Light has been suggested as limiting in other hypereutrophic lakes (Horne 1978; Olrik 1981) and may also restrict N_2-fixation (Horne 1978). Light also has been found to be an important predictor of the relative proportion of blue-green algae in lakes (Smith 1986).

Blue-green algal blooms did not occur in Indian Lake when total N:P ratios were >25, which was similar to Smith's (1983) findings of >29 when he compared percent blue-green algae versus N:P ratios for various lakes. When ratios were >25 in Indian Lake, Chl *a* was generally low. Low N:P ratios also occurred when Chl *a* was low, but the algae were mostly nonblue-greens. Ratios were ~12 during the nonblue-green algal blooms in the spring and fall when Chl *a* was 50–200 $\mu g/L$.

Algae have average elemental ratios of N and P of about 5–7:1 (by weight) (Healey 1973; Wetzel 1983), although differences in cellular ratios can be an important index for indicating algal growth limitations (Healey 1973; Rhee 1978; Kilham and Kilham 1984). For the Indian Lake data, TON and PP were linearly related even as the proportion of blue-green algae increased with increasing Chl *a* levels. The TON:PP ratio by weight (not a true cellular N:P ratio) was 12. This ratio is similar to the total N:P ratio of ~12 when Chl *a* was 50–200 $\mu g/L$ because the dissolved nutrient concentrations were low. The decrease in total N:P ratios (<11) as Chl *a* increased above 200 $\mu g/L$ during the summer blue-green algal blooms was princi-

pally a function of an increase in DIP and algal P levels. During the summer bloom of 1981 when Indian Lake was fertilized, an atypical increase in total N:P ratios occurred because of low DIP, a concomitant reduction in algal PP levels, and an increase in DIN. However, blue-green algae were abundant.

Consequently, low total N:P ratios may only be *predictive* of (or resultant from) blue-green algal blooms rather than *causative*. For the Indian Lake data, P limitation for the spring and fall nonblue-green algae changed to an excess of P for the summer blue-green algae. Ratios decreased as a function of this excess P because DIN was low and TON/Chl *a* was relatively constant for both types of blooms. Canfield (1983) reported a "bending" in the phosphorus–chlorophyll relationship for numerous Florida lakes as TP values increased and as total N:P ratios decreased, which he interpreted as indicating N limitation. This same bending occurred in the Indian Lake data, but the lower ratios probably indicated excess P rather than N limitation.

The Indian Lake results suggest that a long-term decrease in algal biomass in hypereutrophic lakes can only be accomplished by large-scale P control programs that significantly reduce the amount of available P. N fertilization to increase inorganic N concentrations or N:P ratios may not be effective for preventing blue-green algal blooms or sustaining higher N levels because of rapid N loss by denitrification. Based on results from other studies, N fertilization may only be effective for short-term control of N_2-fixing blue-green algae where N limitation is occurring.

Summary

Indian Lake normally had poor water clarity during the summer because of dense blue-green algal blooms typical of hypereutrophic lakes. Levels of total N and P were high because of the algal biomass. DIP was also relatively high but DIN was very low. To test the hypothesis that low DIN levels were a major factor causing the blue-green algal blooms, inorganic N fertilizer was added weekly from late April to early July of both 1981 and 1982.

In 1981, the bloom was similar to other years except that water concentrations of DIP were low and algal P levels were less than normal. In 1982, the water clarity increased in early May, which allowed the proliferation of aquatic macrophytes. Filamentous algae also became very dense, growing on top of the macrophyte community. The clear water was probably a response to the fishkill the previous winter and not a response to the fertilizer. The filamentous algal growth was probably because of the high DIN and DIP levels in the water and good water clarity for growth. The macrophytes may have also provided structural support for the filamentous algae. The macrophytes were again dense in 1983 (posttreatment) but the filamentous algae did not reappear. In both 1982 and 1983 in midsummer, the macrophytes underwent senescence and blue-green algal blooms returned.

The NH_4^+ and NO_3^- from the fertilizer decreased rapidly after each treatment; the NH_4^+ generally had higher depletion rates. Depletion rates for each were similar for both years of fertilization. Apparently, nitrification and denitrification were the main factors controlling the loss of the fertilizer.

Results also indicated that the low total N:P ratios symptomatic of blue-green algal blooms in hypereutrophic lakes were not the result of low DIN, but high levels of DIP that allowed luxury P consumption by the algae. TON:PP ratios, which were analogous to cellular ratios in the algae, were relatively constant for both moderate nonblue-green algal and dense blue-green algal biomasses. When algal biomasses were low, high total N:P ratios were caused by a greater proportion of dissolved inorganic and organic nutrients, particularly DON and sometimes NH_4^+.

In Indian Lake, the major bloom species was *Microcystis aeruginosa*, a non-N_2-fixer. Although DIN was low, the algae were apparently receiving adequate supplies for growth. Evidence also suggests that low DIN did not cause the decline of the spring nonblue-green algae, thus allowing the blue-green algal blooms to develop. Other research studies have shown that blooms of N_2-fixing species such as *Aphanizomenon flos-aquae* may be reduced by N fertilization, but a shift to a bloom of non-N_2-fixing blue-green algae may also occur. However, the rapid loss of the NH_4^+ and NO_3^- makes N fertilization at best a short-term management technique.

Acknowledgements

I thank the numerous people that contributed to this project. Principal field sampling assistants were D. A. Berner, T. L. Brasino, A. M. Journey, K. J. Kramer, S. L. Laumann, P. K. Montz, and R. C. Wildman. G. D. Wegner and G. I. Quinn had a major role in the fertilizer treatments. J. E. Wilken expedited laboratory chemical results needed during the treatments. R. E. Last and P. K. Montz analyzed the phytoplankton samples. Various people, including Drs. B. Boström, D. R. Keeney, and E. E. Prepas, made many helpful suggestions about the manuscript. R. G. Burton produced the figures. The project was funded by the Wisconsin Department of Natural Resources Bureau of Research.

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Domoic Acid — A Neurotoxic Amino Acid Produced by the Marine Diatom *Nitzschia pungens* in Culture

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Subba Rao, D. V., M. A. Quilliam, and R. Pocklington. 1988. Domoic acid — a neurotoxic amino acid produced by the marine diatom *Nitzschia pungens* in culture. Can. J. Fish Aquat. Sci. 45: 2076–2079.

During late 1987, an outbreak of poisoning resulting from the ingestion of cultivated blue mussels (*Mytilus edulis*) from a localized area in eastern Canada (Cardigan Bay, Prince Edward Island) was associated with massive blooms of *Nitzschia pungens*, a widely distributed diatom not previously known to produce toxins; human fatalities resulted. Here we provide proof that the causative agent, domoic acid, is indeed produced by this diatom. Although no domoic acid could be detected (<2 ng·mL^{-1}) in culture medium (FE) prepared from Cardigan River water, it was found in cultures of *Nitzschia pungens* grown in this medium at concentrations ranging from 0.03 to 0.8 pg·cell^{-1} in various separate cultures harvested for chemical analysis 7–68 d after inoculation.

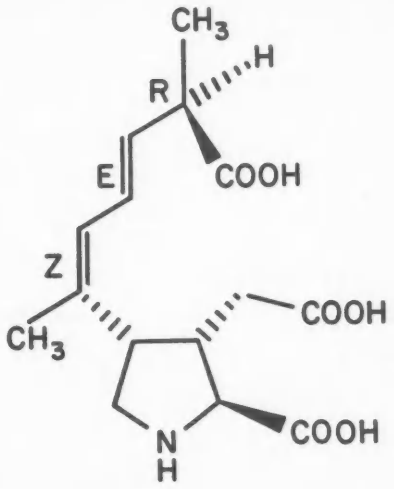
Vers la fin de 1987, un grand nombre de cas d'empoisonnement par suite de l'ingestion de moules bleues cultivées (*Mytilus edulis*) provenant de certaines zones de l'est du Canada (baie Cardigan, Île-du-Prince Édouard) ont été liées à des floraisons massives de *Nitzschia pungens*, une diatomée largement répandue que l'on ne croyait pas produire des toxines. Des décès sont survenus. Les auteurs démontrent que l'agent responsable, l'acide domoïque, est produit par cette diatomée. Bien que l'acide domoïque n'ait pu être décelé (2 ng·mL^{-1}) dans des cultures préparées à partir de l'eau de la rivière Cardigan (milieu FE), cet acide a pu être décelé dans des cultures de *Nitzschia pungens* réalisées dans ce milieu à des concentrations de 0,03 à 0,8 pg·cellule^{-1}, ceci dans diverses cultures distinctes prélevées pour analyse chimique de 7 à 68 jours après l'inoculation.

Received August 3, 1988
Accepted August 22, 1988
(J9826)

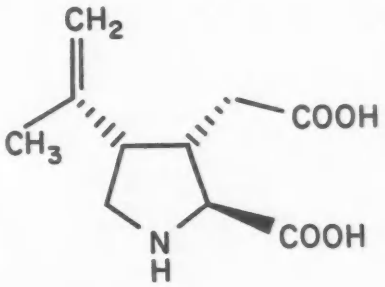
Reçu le 3 août 1988
Accepté le 22 août 1988

Domoic acid (**1**) is a naturally occurring amino acid previously isolated from two red algae (*Chondria armata* and *Alsidium corallinum*) (Fattorusso and Piattelli 1980) but unknown in the phytoplankton. It is an excitatory amino acid and probably acts as an agonist to glutamate, a neurotransmitter in the central nervous system, in a manner analogous to kainic acid (**2**) (Hampson and Wenthold 1988). This toxin was found in cultivated blue mussels (*Mytilus edulis*) harvested November 1987 from Cardigan Bay in eastern Prince Edward Island, Canada (J. L. C. Wright et al., submitted). The three fatalities and over 100 cases of sickness that occurred from the ingestion of these mussels were attributed to domoic acid poisoning. Analyses of aqueous extracts of the whole mussel meats gave concentrations (up to 900 μg·g^{-1} in the mussel tissue) sufficient to induce toxic symptoms in humans. Gut contents of mussels from the Cardigan River, P.E.I., where the toxicity was most acute, revealed a preponderance of *Nitzschia* sp. Water samples and concentrates of phytoplankton showed a monospecific bloom of a chain-forming *Nitzschia* sp. (99% of total phytoplankton) in concentrations of ~10^7 cells·L^{-1} comparable with those found in "red-tide" blooms. The chains of this *Nitzschia* sp. were stiff, motile, and had a maximum of 39 cells per chain. The cells were 80–140 μm long, 4.5–6 μm wide, symmetric, spindle-shaped with pointed ends, and contained two chromatophores (Fig. 1). The species was identified as *Nitzschia pungens* Grunow f. *multiseries* Hasle (Hasle 1965) by M. Poulin (National Museums of Canada, Ottawa, Ont., pers. comm.) and C. Bird (ARL, pers. comm.) and was confirmed by G. R. Hasle (University of Oslo, Norway).

Domoic acid was found in the Cardigan River blooms of *Nitzschia pungens* providing circumstantial evidence that the source of domoic acid in the mussels was the *Nitzschia pungens* consumed by the mussels (S. Bates et al., in prep.). The question of whether the toxin was produced *de novo* by *Nitzschia* itself or that the diatom was merely a vector could not be properly addressed until large quantities of the organism could be cultured. Single chains of *Nitzschia pungens* cells were isolated from Cardigan Bay water sampled on 16 December 1987, using a modified 1-mL hypodermic syringe fitted with a finely drawn microhaematocrit tube. They were washed several times with sterile medium to minimize transfer of contaminating bacteria and other microorganisms. Cells were grown at 10°C and



1



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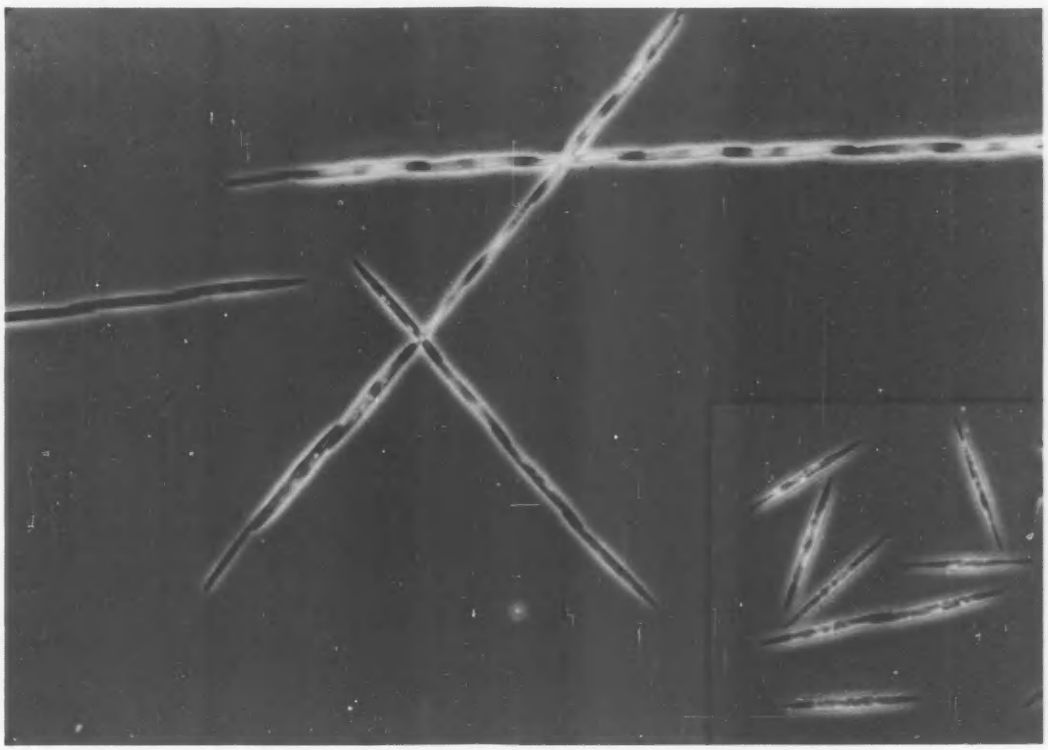

FIG. 1. Chains of *Nitzschia pungens* in culture with some individual cells inset. ×500.

exposed to 165 $\mu E \cdot m^{-2} \cdot s^{-1}$ continuous fluorescent light in sterile medium FE (Subba Rao 1980), with aged (mid-December 1987 and mid-January 1988) Cardigan Bay water enriched with F nutrients (Guillard and Ryther 1962), plus 2% (v/v) Cardigan Bay region soil extract fortified with 4 g $NaNO_3 \cdot L^{-1}$ and 0.6 g $Na_2HPO_4 \cdot 12H_2O \cdot L^{-1}$. Samples preserved with neutral Lugol solution were enumerated under an inverted plankton microscope. The division rates of our cultures ranged from 0.77 to

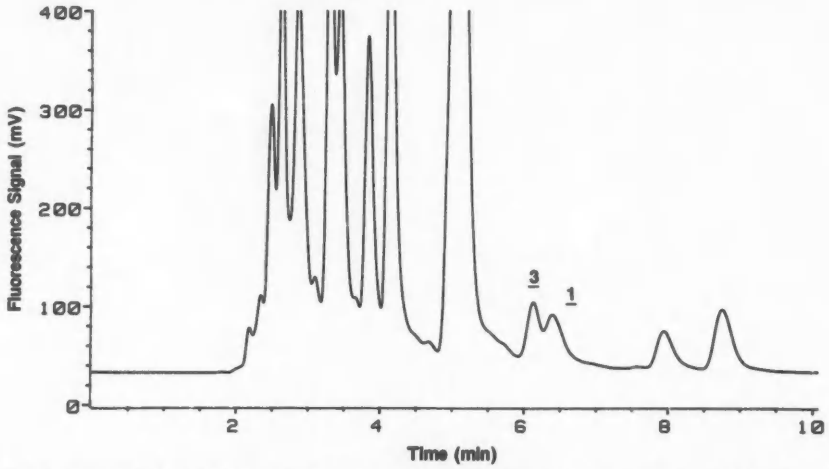

FIG. 2. Reversed-phase HPLC analysis of the FMOC derivatives of the compounds extracted from cultured *Nitzschia pungens* isolated by filtration. The FMOC derivative of domoic acid (**1**) elutes after proline (**3**) in the sequence of natural amino acids. Sample (200 μL) and borate buffer (50 μL; pH 6.1) were mixed in a 1-mL reaction vial. The reagent (250 μL of a 15 mM solution of 9-fluorenylmethyl-chloroformate in acetonitrile) was added and the mixture agitated. After 45 s, the excess reagent was removed by extraction into ethyl acetate (3 × 250 μL). Aliquots (5 μL) of the aqueous layer were injected onto a 5-μm Vydac 201TP column (25 cm × 2.1 mm inside diameter) with 0.3 mL·min^{-1} 40% aqueous acetonitrile and 0.1% trifluoroacetic acid. A fluorescence detector was used with an excitation wavelength of 265 nm and emission wavelength of 305 nm.

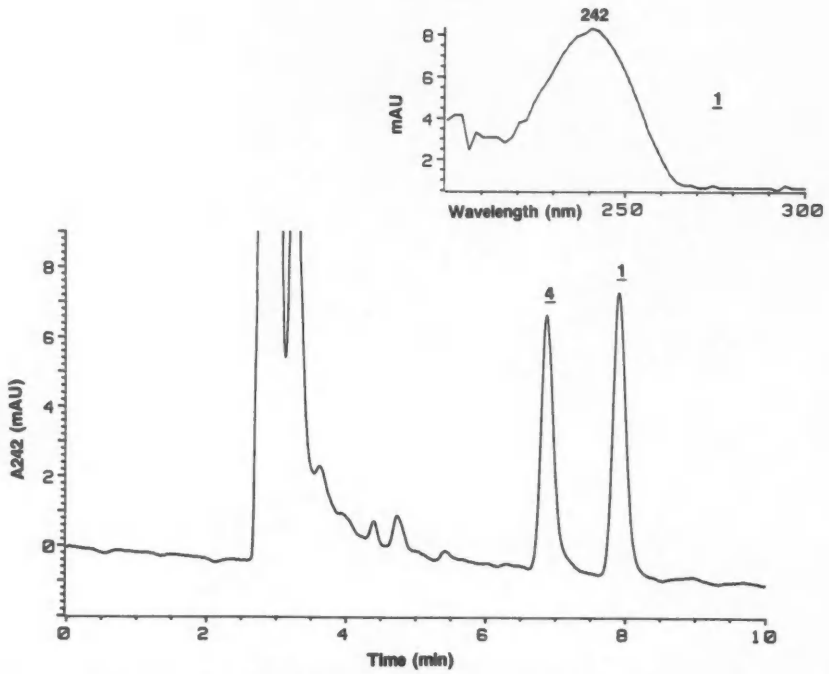

FIG. 3. Reversed-phase HPLC-DAD analysis of an extract of cultured *Nitzschia pungens* isolated by filtration. A 5-μm Vydac 201TP column (25 cm × 4.6 mm inside diameter) was operated with 1 mL·min^{-1} 10% aqueous acetonitrile and 0.1% trifluoracetic acid. Detection at 242 nm was used to generate the chromatogram and the insert shows the UV absorption spectrum acquired at the apex of the domoic acid (**1**) peak. The preceding peak is tryptophan (**4**).

$0.80 \cdot d^{-1}$ (mean = $0.79 \cdot d^{-1}$) and compare favorably with division rates of other marine diatoms (Subba Rao 1981).

Determination of domoic acid directly in extracts of the whole culture and in filtrates of the culture was by a high-sensitivity (nanograms per millilitre) modification of the 9-fluorenylmethoxycarbonyl chloride (FMOC) precolumn derivatization method for amino acids (Einarsson et al. 1986) followed by reversed-phase HPLC with fluorescence detection (HPLC-FD). Recognition of domoic acid was by coincidence of retention time with that of authentic standard. There was no detectable domoic acid (<2 ng$\cdot$mL^{-1}) in the sterile FE medium prior to inoculation. At various times after inoculation, samples (usually 200 mL) at cell densities ranging from 2×10^4 to 4×10^4 cells$\cdot$mL^{-1} were filtered, and after extraction (methanol–water, 1:1), reduction in volume, and removal of interfering substances (C-18 solid phase extraction), domoic acid was determined in the cells filtered from solution by both the FMOC/HPLC-FD method (Fig. 2) and by direct analysis with HPLC followed by diode array detection (HPLC-DAD) (M. A. Quilliam et al., submitted) which additionally provided UV spectra (Fig. 3).

The first evidence that domoic acid is produced by *Nitzschia pungens* in culture was obtained on 12 April 1988. This was further established when a larger quantity ($\sim 10^7$ cells) was filtered and the presence of domoic acid in the cells on the filter confirmed by both HPLC methods supported by the UV spectrum. After preparative reversed-phase HPLC was performed on an extract of $\sim 10^9$ cells of *Nitzschia pungens* harvested 3 May 1988, full confirmation of the identity of domoic acid was performed by matching the proton NMR spectrum with that of domoic acid isolated from *Chondria armata* (Ohfune and Tomita 1982) and toxic mussels from Cardigan Bay (J. L. C. Wright et al., submitted).

Cellular domoic acid concentration varied widely from one batch culture to another, but generally increased with the age of the culture. For example, in various separate cultures harvested for chemical analysis, the domoic acid concentration was measured at 0.03, 0.2, and 0.8 pg$\cdot$cell^{-1} after 7, 17, and 68 d of growth, respectively. Concentrations of domoic acid in the medium did not exceed the FMOC/HPLC-FD detection limit (2 ng$\cdot$mL^{-1}); at least 85% of the total domoic acid was in the cellular fraction. These concentrations approach those encountered in December in samples of phytoplankton collected in eastern Prince Edward Island waters (S. Bates et al., in prep.).

The environmental conditions in eastern Prince Edward Island in October–December 1987 that stimulated the growth of this diatom cannot be established, but *Nitzschia pungens* cultured in the local seawater certainly produces domoic acid. The finding that cultures of *Nitzschia pungens* produce domoic acid *de novo* makes more credible the argument that blooms of this diatom were responsible for the toxicity of the mussels in Cardigan Bay. There are obvious public health implications to the presence of a known neurotoxin in such a widely distributed diatom.

Acknowledgments

We acknowledge the valuable contributions of Carolyn Bird (ARL) in the identification of the *Nitzschia* species, John Walter (ARL) for provision of the NMR spectrum, and the technical support of N. Crewe (BIO), C. Craft (ARL), and W. Hardstaff (ARL).

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Woody Debris and Its Contribution to Pool Formation in a Coastal Stream 50 Years after Logging[1]

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Large quantities of woody debris persisted 50 yr after logging and fire in stream channels of a small coastal Oregon watershed. Debris from the current stand represented only 14% of total debris volume and 8% of debris volume responsible for creating pools. The greatest number of pools were located in downstream sections of the watershed where gradient was reduced, discharge was increased, and streambed material was finer. Seventy percent of pools with a volume greater than 1.0 m^3 were associated with woody debris in the channel. Composition of the current riparian forest varied with topography. Alder stands dominated moist terrace sites adjacent to channels, whereas slopes contained a mixture of alder and conifer. Study results indicate that riparian trees must be left to grow longer than 50 yr to ensure that an adequate, long-term supply of woody debris is available to stream channels. Debris from previous stands plays a crucial role in the interim and should not be removed from stream channels.

D'importantes quantités de débris ligneux occupaient toujours les chenaux de cours d'eau d'un petit bassin versant côtier de l'Oregon, 50 ans après l'exploitation forestière et la destruction par le feu de cette région. Les débris provenant du peuplement actuel ne constituent que 14 % du volume total des débris et 8 % du volume de ces débris ont contribué à la création de mouilles. Les sections d'aval du bassin versant où le gradient était réduit, le débit plus élevé et les matériaux du lit plus fins, comportaient le plus grand nombre de mouilles. Soixante-dix pour cent des mouilles dont le volume dépassait 1,0 m^3 étaient associées à la présence de débris ligneux dans le chenal. La composition de la ripisilve actuelle variait selon la topographie. Des peuplements d'aulnes dominaient les terrasses humides adjacentes au chenal, tandis que sur les pentes poussait un mélange d'aulnes et de conifères. Les résultats de l'étude révèlent qu'il faut laisser croître les arbres poussant en bordure des cours d'eau pendant plus de 50 ans pour s'assurer un approvisionnement à long terme adéquat en débris ligneux dans les chenaux. Les débris provenant de peuplements antérieurs jouent un rôle vital dans l'intervalle et ne devraient pas être éliminés du chenal des cours d'eau.

Received February 11, 1988
Accepted August 12, 1988
(J9586)

Reçu le 11 février 1988
Accepté le 12 août 1988

Decaying wood that litters the channels and banks of streams flowing through forested land plays important roles in structuring stream channels. For example, wood in stream channels diverts and obstructs streamflow, thereby creating a more complex arrangement of pools and other channel features than would exist without the debris (Bisson et al. 1987). This channel complexity is particularly important for fish species that use pools and gravel deposits for spawning and rearing their young.

The amount of woody debris in streams is, in part, a product of the surrounding forest. Healthy young stands typically produce less woody debris than do the older stands they replace (Grette 1985). Woody debris from streamside forests enters stream channels as a result of high winds, fire, disease, and water undercutting tree roots. In steep terrain, landslides also move woody debris from adjacent slopes to stream channels.

The amount of woody debris in coastal Oregon streams has been altered over the past 80 yr as a result of logging, road building, and the conversion of forested land to other uses. Decades ago, the combination of downhill logging to stream channels and low wood utilization increased the load of woody debris in some coastal streams (Gharrett and Hodges 1950, cited in Sedell and Luchessa 1982). In some streams the amount of debris was reduced when water stored behind temporary dams was released to flush logs and cedar bolts downstream. Wood has been removed from streams to protect bridges and culverts from damage during floods and from coastal lowland streams channelized to make way for pasture. In addition, woody debris

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has recently been cleared from some streams on forested land in a misguided effort to enhance fish productivity (Bilby 1984).

Supplies of woody debris available to stream channels can be reduced by short harvest rotations combined with high wood utilization. Because such reductions can adversely affect stream systems, current rules for forest practices in the state of Oregon require that riparian zones of logged watersheds be maintained in a condition that enables them to provide both shade and woody debris to streams.

The objective of the study reported here was to determine how young stands of timber might be managed so that they supply adequate amounts of woody debris to fish-bearing streams. To assess the adequacy of the supply of woody debris in a fourth-order coastal Oregon stream system, we measured the amount of debris that remained from a previous stand subjected to logging and fire and the amount that originated in the current stand. We then examined the relative contribution of old and new woody debris to pool formation in sections of the main stem. In addition, we measured the basal area and stem density of streamside forest and examined how these varied throughout the watershed.

Methods

Watershed Characteristics

Big Creek (Lincoln County, Oregon) afforded a unique opportunity to document not only the management history, but also the resulting riparian zone characteristics of a fourth-order stream. The portion of the watershed studied drains 6.5 km^2 and lies within 1–6 km of the Pacific Ocean, into which the stream eventually flows (Fig. 1). It is managed by the Siuslaw National Forest. The downstream section (A) of Big Creek is relatively flat and flows through marine terrace basalts and siltstone. An 8-m waterfall separates the upstream from the downstream section. Low-flow discharge at the downstream end of the study area is about 0.2 m^3/s. The upstream basin is steep-sided basalt. Except for disturbance by landslides in section C, sections B and C are quite similar. Other sections sampled included section D on the main stem; section E (tributaries 1, 2, and 3); section F (tributary 4), a headwater stream disturbed by torrents; and section G (tributary 5 and 6), headwater streams not disturbed by torrents. Average annual precipitation is 180 cm.

Except for a small section of the upstream basin, the Big Creek watershed has not been logged since a wildfire swept through the area 50 yr ago. Most of the basin was clearcut prior to the fire and revegetated naturally afterwards. Certain upslope areas were planted with conifers in the 1940s. Streams in the watershed are now bordered by a dense stand of trees and shrubs, including red alder (*Alnus rubra*), salmonberry (*Rubus spectabilis*), Sitka spruce (*Picea sitchensis*), western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and Douglas-fir (*Pseudotsuga menziesii*). In the partially logged upstream portion of the watershed, trees that survived the fire exist today amid the second-growth forest.

A variety of other disturbances have occurred in the watershed. Aerial photographs indicate that between 1928 and 1936, two railroad spurs were used to haul logs and quarry rock to the coast (see Fig. 1). The railroad grade along the main stem of Big Creek encroached on the stream channel in several places in section D. Woody debris from downhill cable logging to railroad landings accumulated in or near the stream channel. Landslides in a section of Big Creek watershed were initiated

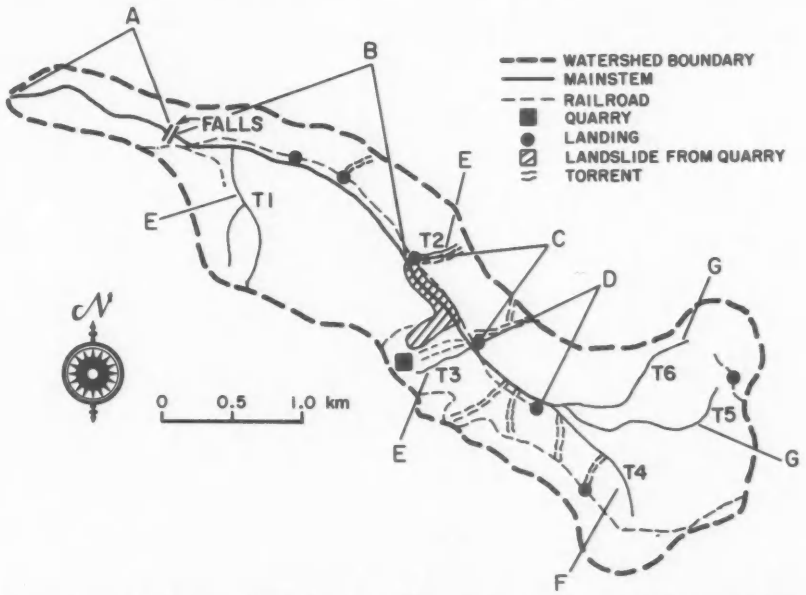

FIG. 1. Study area located in Big Creek watershed (Lincoln County, Oregon). Sections are labeled as follows: A = main stem, downstream from falls, sedimentary geology; B = main stem, segments not disturbed by landslides; C = main stem, segments disturbed by landslides; D = main stem, railroad grade close to stream channel; E = tributaries 1, 2, and 3, enter main stem in sections B and C; F = headwater tributary, very steep slopes, disturbed by torrents; G = headwater tributaries, not disturbed by torrents.

TABLE 1. Stream characteristics and woody debris volume in Big Creek watershed.

Measurement	Stream section						
	A	B	C	D	E	F	G
Length sampled (m)	1550	1950	750	750	2200	1050	2900
Width (m)							
Low-flow	3.3	2.7	2.3	2.6	0.9	1.6	1.0
Bankfull	7.8	6.1	5.9	5.5	3.4	2.9	2.5
Gradient (%)	2.2	4.9	5.7	6.0	13.3	12.7	12.5
Volume of woody debris (m^3/100 m)							
From previous stand	14.9	13.0	20.4	28.0	24.4	57.6	33.2
From current stand							
Hardwood	3.2	1.3	2.0	4.3	1.2	2.8	2.6
Conifer	0.0	1.0	0.8	0.5	2.4	4.0	3.8
Total	18.1[a]	15.3	23.2	32.8	28.0	64.4	39.6
Current stand debris (% of total)	18	15	12	15	13	11	16

[a]Does not include a large (99 m^3), stable accumulation of woody debris at base of falls.

TABLE 2. Position of old and new debris (%) in Big Creek main stem and tributaries.

Position	Main stem		Tributaries	
	Old debris	New debris	Old debris	New debris
In channel	40	19	18	7
Suspended above channel	15	35	37	43
On banks or terraces	45	46	45	50

by failure of spoil piles at a rock quarry from about 1950 to 1961; two unrelated landslides occurred around 1969. In addition, small debris avalanches and torrents have occurred in some tributaries, including section F, within the past 50 yr. Debris torrents begin as small landslides into a steep channel. Subsequently, material moving down the channel picks up additional soil, water, and woody debris. The torrent commonly deposits its load when it reaches a lower gradient stream.

Stream and Stand Measurements

The Big Creek main stem and six tributaries in which debris torrents provide potential sources of wood for the main stem were sampled. Channels were marked at 50-m intervals, where channel and valley floor characteristics were noted. Woody debris greater than 10 cm in diameter and 1 m in length was inventoried in a strip 5 m wide on either side of a centerline along the entire channel. For some pieces, diameter and length were measured; the size of other pieces was estimated visually. Periodic measurements of estimated lengths or diameters helped to maintain estimation accuracy. Each piece of debris was classified by origin (previous or current stand). Piece diameter, level of decay, and species were used as indicators of origin. All hardwood debris was assumed to be from the current stand. The position of each piece was also determined. Position categories included suspension above the channel, in the active channel, and on the banks and terraces. If a piece occupied more than one position within the 10-m-wide strip, it was assigned the position that corresponded with most of the piece volume (Long 1987).

Pool depth and volume were determined in every third 50-m interval in the main stem. Only pools at least 1.0 m^3 in volume and 30 cm in average depth were included in the analysis. Each of the sampled pools was assigned a primary reason for formation, and contributing factors (e.g. banks stabilized by roots and bedrock depressions) were documented. Wood pieces in pools created by woody debris were measured and classified.

Seventy-seven streamside forest plots, spaced at 100- or 150-m intervals along the main stem and tributaries, were also sampled. Each plot paralleled the stream centerline for 10 m and extended 20 m on either side, thus containing an area of 400 m^2 minus the area of the stream channel. Plot areas were categorized according to landform (slope or terrace). In each plot, species and diameter at breast height (DBH) were determined for all trees, both live and dead, greater than 15 cm DBH.

Results

Woody Debris from the Previous Forest

Debris originating in the previous stand (old debris) was abundant in sections C and D of the main stem, where the railroad grade paralleled and occasionally encroached upon the stream (Table 1). Downhill cable logging to streamside landings created large accumulations of wood in the channel. Some of this wood was later buried or piled by landslides. Landslides also moved hillside woody debris into the channel, and large accumulations of woody debris were commonly found on the downstream edge of landslide deposits.

Old debris was least abundant in sections A and B of the main stem (see Fig. 1). The railroad grade was not located near the stream in this area. In addition, slopes bordering the stream were moderate and this decreased the likelihood that trees cut on upslope areas rolled into the stream. Only small amounts of woody debris occurred in a bedrock section extending 200 m upstream from the falls. A large accumulation of debris at the base of the falls indicated that high water velocity in this constricted bedrock section moved debris over the falls. Large logs apparently were not moved by high flows in the low-gradient section downstream from the falls.

Overall, old debris in the main stem measured 16.9 m^3/100 m or 1.7 m^3/100 m^2. This is about half Grette's (1985) mean value of 3.9 m^3/100 m^2 measured for six coastal Washington streams bordered by stands 40–62 yr of age.

The volume of old debris in the tributaries was about twice that in the main stem (34.2 compared with 16.9 m^3/100 m).

TABLE 3. Numbers of pools originating from various causes in the main stem of Big Creek (standard deviations are in parentheses).

Cause	Importance in pool-forming process[a]	Numbers of pools: Stream section A	B	C	D	Total	Average pool volume (m^3)
Pools caused by debris							
Flow over top of log	P	6	6	0	2	14	4.8 (3.2)
(log step)	S	0	0	0	0	0	
Channel constricted	P	4	4	2	1	11	2.9 (1.5)
by debris	S	9	5	0	2	16	
Flow underneath debris	P	9	0	0	0	9	3.8 (2.0)
	S	1	0	0	0	1	
Flow diverted by debris	P	6	0	1	0	7	3.7 (1.1)
into streambank	S	2	1	0	0	3	
Flow against upturned	P	0	2	0	0	2	5.3 (2.9)
rootwad in channel	S	0	0	0	0	0	
Total	P	25	12	3	3	43	
	S	12	6	0	2	20	
Pools not caused by debris							
Flow into streambank	P	9	1	0	0	10	6.0 (5.7)
(no debris)	S	1	1	0	0	2	
Bedrock depression	P	0	7	0	0	7	3.0 (1.5)
	S	0	0	0	0	0	
Large rocks from	P	0	0	1	0	1	1.8
quarry landslide	S	0	0	2	0	2	
Total	P	9	8	1	0	18	
	S	1	1	2	0	4	

[a]P = primary, S = secondary.

Steep slopes and the absence of a terrace between the channel and adjacent slopes increased the likelihood that debris would roll into the tributary channels. Also, there were more large conifer stumps next to the tributary channels than next to the main stem channel. Terraces along the main stem channel were presumably bordered by an alder and brush stand, the debris and stumps of which would have already decayed. Large accumulations of old debris in the tributaries can probably be attributed to steep slopes and the predominance of conifers.

The volume of old debris in section F was from 1.7 to 4.4 times greater than that in the other sections sampled. Section F flowed through unusually steep terrain, and torrents deposited woody debris from unslope areas into the channel at two locations. There was no evidence that high discharges apart from torrents caused debris movement.

Woody Debris from the Current Forest

Of the total debris volume, only 14% originated in the current forest (new debris). New debris volume averaged 3.0 m^3/100 m in the main stem and 5.5 m^3/100 m in the tributaries.

Conifers were more abundant next to the tributaries than they were next to the main stem. Consequently, the volume of new conifer debris was over five times greater in the tributaries than in the main stem. The volume of new hardwood debris in the tributaries was similar to that in the main stem. Hardwood debris volume in the main stem was greatest in sections A and D (Table 1), where dense streamside alder stands occurred. Much of the addition of new hardwood debris in section A resulted from undercutting of tree roots by water. In section D, windthrow of streamside alder growing on boggy terraces was a common cause of debris input. Correspondingly less new hardwood debris occurred in the remaining main-stem reaches, which were bordered either by young stands that originated after landslides or by stands of low density.

Differences between Old and New Woody Debris

Debris was positioned in the channel, above the channel, and on the banks and terraces (Table 2). Only 19% of new debris in the main stem and 7% of new debris in the tributaries resided in the channel. A larger percentage of the old debris was found within the channel for both main stem and tributaries. Average piece volume of old and new debris was 0.93 and 0.26 m^3, respectively. Piece diameter of old debris averaged twice that of new debris (45 cm compared with 24 cm). Some pieces of old debris were as large as 200 cm in diameter, but most (95%) fell within a range of from 10 to 90 cm. The diameter of most pieces of new debris (97%) ranged from 10 to 40 cm.

In general, old debris was more decayed than new debris. However, often the rate of decay was affected more by debris species and location than by the length of time it had been decomposing. For example, cedar logs from the previous stand were usually firm, with only a rind of decay, whereas some 10-yr-old alder debris from the current stand was soft and punky. Debris buried by sediment or covered by water appeared to be more sound than debris exposed to air.

Pools

Individual pool volume ranged from 1 to 19 m^3, and the largest pools were located in the downstream reaches of the main stem. Seventy percent of the pools in the main stem were created primarily by woody debris; the remainder resulted from water directed at a stable streambank, scouring around large rocks, or bedrock depressions (Table 3). Despite the lower vol-

TABLE 4. Characteristics of pools[a] and woody debris contributing to pool formation in Big Creek (standard deviations are in parentheses).

Measurement	Stream section A	B	C	D
Length of stream sampled (m)	582	627	258	355
Pools				
Number	34	20	4	3
Frequency (No./100 m)				
Formed primarily by woody debris	4.3	1.9	1.2	0.8
Formed by other processes	1.6	1.3	0.4	0.0
Volume (m^3/100 m)				
Formed primarily by woody debris	17.8	7.7	3.2	2.9
Formed by other processes	9.1	4.4	0.7	0.0
Size				
Width (m)	2.1 (1.2)	2.9 (1.2)	1.8 (0.2)	2.9 (0.9)
Length (m)	4.7 (2.7)	3.0 (1.6)	3.3 (0.9)	2.5 (0.2)
Maximum depth (m)	0.66 (0.11)	0.62 (0.15)	0.60 (0.11)	0.63 (0.11)
Average depth (m)	0.50 (0.06)	0.46 (0.07)	0.42 (0.05)	0.45 (0.08)
Area (m^2)	9.2 (7.1)	8.1 (3.9)	5.9 (0.9)	7.3 (2.9)
Volume (m^3)	4.6 (3.8)	3.8 (2.1)	2.5 (0.5)	3.4 (1.7)
Woody debris				
Volume per pool[b] (m^3)	3.4 (3.9)	3.4 (2.1)	2.6 (4.2)	6.8 (1.7)
Pieces per pool[b] (no.)	3.9 (2.7)	2.8 (1.7)	2.3 (2.1)	5.3 (2.5)
Average piece volume[b] (m^3)				
From previous stand	1.28 (2.36)	1.23 (1.19)	1.31 (1.72)	1.35 (1.13)
From current stand	0.16 (0.25)	0.74 (0.74)	0.06[c]	0.11[c]
Volume contributing to pool formation (m^3/100 m)				
From previous stand	14.1	6.5	3.0	5.7
From current stand	1.2	0.8	0.0	0.0

[a]Includes only pools ≥1 m^3 in volume and ≥30 cm in average depth.
[b]Includes only pools caused primarily by woody debris and those pieces of debris contributing to pool formation.
[c]Sample size = 1.

ume of woody debris in sections A and B, debris-formed pools were two to five times more numerous in section A than in upstream sections of the main stem (Table 4). This is probably because discharge was greater and streambed material was finer downstream from the falls. In addition, the low-gradient channel in the downstream section meandered freely across a wide valley floor, thus providing more opportunities for woody debris to create pools than occurred upstream. Debris commonly combined with a stable bank to create pools at bends in the downstream section.

Most of the pools created by debris were formed by old debris. Only 7% of the debris volume responsible for creating pools originated in the current stand. The average size of old pool-forming debris ranged from 1.23 to 1.35 m^3, whereas the size of new pool-forming debris ranged from 0.11 to 0.74 m^3, depending on main stem section (Table 4). On the average, three pieces of wood were associated with the formation of each pool. Often a single piece of debris created the major flow diversion, and other pieces constricted flow upstream or controlled pool boundaries. No correlation was found between the volume of debris-formed pools and total debris volume (per 50-m reach). Furthermore, no correlation existed between pool volume and the portion of total debris identified as contributing to pool formation. This suggests that, beyond some threshold level, factors other than debris abundance are responsible for the amount of pool volume per unit length of stream.

Current Riparian Forest

The streamside forest varied considerably with landform. The terrain sampled was 48% slope, 42% terrace, and 10% terrace disturbed by landslides. There were no terraces along the tributaries. Most of the terrace trees along the main stem were alder (89% of total basal area), whereas adjoining slopes supported a mixture of alder and conifer (mostly Sitka spruce and hemlock). Forests along tributaries contained a greater proportion of conifer (82% of the total basal area), although total basal area of tributary stands was similar to that of stands along the main stem. Terraces disturbed by landslides supported only half the basal area of undisturbed areas, reflecting the younger stand age.

No correlation was found between stand volume in sample plots and the volume of new debris in encompassed 50-m reaches. Although variation among sample plots was high, average stand density on plots in different sections of the watershed was similar (Fig. 2). Stand density was particularly variable along the main stem where salmonberry and other brush species often occupied large patches and limited or eliminated tree establishment. Ten percent of the terraces and 5% of the slopes along the main stem supported only brush.

Standing dead trees (snags) from the current stand were notably scarce (9 snags/ha along the main stem; 5 snags/ha along tributaries). Alder snags only (average DBH = 21 cm) were found along the main stem, whereas conifer snags (average DBH = 35 cm) occurred near tributary channels.

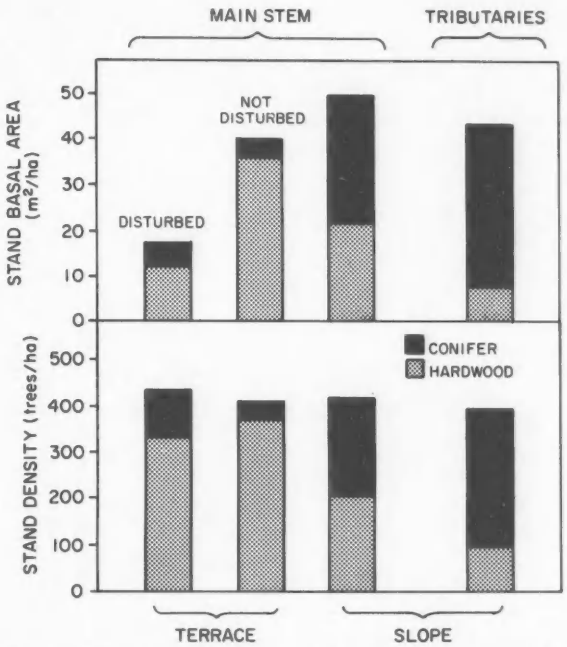

FIG. 2. Characteristics of riparian stands in the Big Creek watershed. Disturbed terraces in the main stem were a result of landslides.

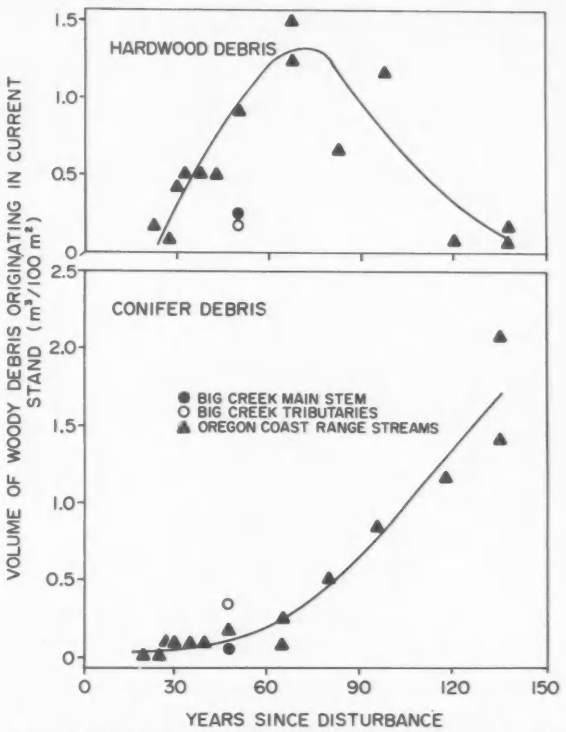
HARDWOOD DEBRIS
CONIFER DEBRIS
VOLUME OF WOODY DEBRIS ORIGINATING IN CURRENT STAND (m³/100 m²)
BIG CREEK MAIN STEM
BIG CREEK TRIBUTARIES
OREGON COAST RANGE STREAMS
YEARS SINCE DISTURBANCE

FIG. 3. Comparison of new debris in Big Creek main stem and tributaries with levels of debris in other Oregon Coast Range streams D. C. Heimann, U.S.G.S., Kansas City, MO., unpubl. data).

Discussion

Results from this study indicate that trees must grow beyond 50 yr before riparian stands yield large debris in quantities similar to old-growth forests; however, the amount of woody debris essential for maintenance of the present pool frequency in a fourth-order stream system remains unclear. Of the total woody debris volume, 85% contributed to pool formation in section A, but only 15% contributed in sections C and D. Stream characteristics such as gradient and bedload composition appear to be important determinants in the formation of debris-associated pools.

The volume of new debris, both conifer and hardwood, in the main stem of Big Creek was smaller than that in other streams of similar size and stand history in the Oregon Coast Range (Fig. 3). Also, Grette (1985) showed that new debris in coastal Washington stream channels bordered by 40- to 62-yr-old stands averaged 0.76 $m^3/100\ m^2$; this is more than twice that found in the Big Creek main stem ($0.30\ m^3/100\ m^2$). During the next two decades, additions of new debris to Big Creek channels should increase substantially, and in a pattern similar to that shown in Fig. 3. Few conifer seedlings or saplings occur among alder stands on terraces because salmonberry and alder tend to occupy openings; therefore, future conifer debris in the main stem will originate mainly in forests on adjoining slopes. In addition, debris suspended above the channel will decay, enter the stream, and influence pool formation. Old debris, much of it already in advanced stages of decay, will contribute less and less to channel complexity.

Oregon Forest Practices Rules (August 1987) address the need for large, stable debris in the stream channel. The rules require that landowners harvesting timber next to class I streams (e.g. Big Creek) leave enough vegetation to maintain 75% of prelogging shade over the aquatic area and 50% of the prelogging canopy in the riparian area. In addition, landowners must leave an average of at least 22 live conifers/ha and a conifer basal area of 2.3 m^3/ha in the riparian management area. All snags that are not a safety or fire hazard and all downed wood in the riparian area must be left intact. By requiring that conifers be left next to streams, Oregon law recognizes the need for long-term presence of large, stable debris. Sections of Big Creek lack even 22 conifers/ha as prescribed by the rules. Specific efforts may be needed to ensure establishment of conifer seedlings in riparian areas currently lacking them and where inadequate long-term supplies of debris can be demonstrated. Planting conifers within riparian areas (with necessary brush control and attention to water quality concerns) may be an appropriate management strategy.

Acknowledgments

We appreciate the help provided by the Waldport Ranger District, USDA Forest Service, in unraveling the history of the Big Creek watershed.

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Response of Wild Rainbow (*Salmo gairdneri*) and Cutthroat Trout (*S. clarki*) to Stocked Rainbow Trout in Fertile and Infertile Streams[1]

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Petrosky, C. E., and T. C. Bjornn. 1988. Response of wild rainbow (*Salmo gairdneri*) and cutthroat trout (*S. clarki*) to stocked rainbow trout in fertile and infertile streams. Can. J. Fish. Aquat. Sci. 45: 2087–2105.

Wild rainbow trout (*Salmo gairdneri*) and cutthroat trout (*S. clarki*) were unaffected by stocking of catchable-size rainbow trout in two Idaho streams, except at the highest stocking rates, and even then the effects were limited. In the infertile stream, stocking 50 or 150 trout per section (doubling or tripling the density) did not reduce the abundance of wild cutthroat trout. Wild trout abundance declined at a faster rate in an unreplicated section stocked with 500 trout than in unstocked sections. In the fertile stream, stocking 50 or 100 hatchery trout in sections containing 26–120 similar-sized wild trout did not increase the dispersion or reduce the abundance, growth, or survival rates of wild rainbow trout. When we stocked 400 trout (100 on four dates) in sections containing 32–53 tagged wild trout of similar size, the summer mortality rate of wild trout was higher in stocked than in unstocked sections; the other parameters were not significantly different.

On a constaté dans deux cours d'eau de l'Idaho que la truite arc-en-ciel (*Salmo gairdneri*) sauvages et la truite fardée (*Salmo clarki*) sauvages ne sont pas gênées par l'arrivée de truites arc-en-ciel d'ensemencement de taille légale, sauf aux rythmes d'ensemencement les plus élevés, et que même dans ces conditions les effets sont limités. Dans le cours d'eau infertile, l'ensemencement de 50 ou 150 truites par section (ce qui double ou triple la densité) n'a pas réduit l'abondance de la truite fardée sauvage. L'abondance de la truite sauvage a diminué plus rapidement dans une section sans équivalent où l'on a déversé 500 truites que dans les sections non ensemencées. Dans le cours d'eau fertile, l'ensemencement de 50 ou 100 truites d'élevage dans des sections où se trouvaient de 26 à 120 truites sauvages de même grosseur n'a pas eu l'effet de disperser davantage les truites arc-en-ciel sauvages ni de réduire leur abondance, leur taux de croissance ou leur taux de survie. Lorsque nous avons ensemencé 400 truites (100 truites, quatre jours différents) dans des sections où il y avait de 32 à 53 truites sauvages étiquetées de grosseur semblable, le taux de mortalité de la truite sauvage en été était plus élevé dans les sections ensemencées que dans les sections non ensemencées; dans le cas des autres paramètres, il n'y avait pas de différence significative.

Received May 14, 1986
Accepted August 10, 1988
(J8795)

Reçu le 14 mai 1986
Accepté le 10 août 1988

Stocking of catchable-size (>15 cm) rainbow trout (*Salmo gairdneri*) was studied in two Idaho streams to determine if hatchery trout would negatively affect the wild trout present. The abundance, distribution, and behavior of native cutthroat trout (*Salmo clarki*) was assessed in the St. Joe River, a relatively infertile stream, in the summer of 1979 before and after stocking of hatchery rainbows. A naturally reproducing population of rainbow trout was studied in a productive stream (Big Springs Creek) during 1979–82 to determine if the stocked rainbows survived and if they altered the abundance movements, survival rates, and growth of the wild fish.

Hatchery trout have been stocked in streams for decades to supplement the harvest available from populations of native or feral trout. Native populations of trout have declined in many streams stocked with hatchery trout, in some cases before the stocking and in some coincident with stocking (Johnson and Bjornn 1978). In some streams, introduced trout are now found in place of the native trout. In most cases, it is not clear if the introduced trout displaced the native trout or if they merely occupied habitat no longer used by native trout because of excessive exploitation.

In early studies of native versus introduced trout stocks (Needham and Slater 1944; Miller 1954, 1958; Reimers 1957), evidence was presented that introduced trout were at a competitive disadvantage, as might be expected from commonly accepted fitness and behavior theory. Native trout would be better adapted to stream conditions than introduced trout and have a prior residence behavioral advantage. In more recent literature (Clady 1973; Butler 1975; Reisenbichler and McIntyre 1977; Krueger and Menzel 1979; Vincent 1972, 1975, 1987), however, investigators have hypothesized that competition for food and space between wild and hatchery trout may be severe.

In the experiments reported here, we tested for direct competitive effects of stocked catchable-size rainbow trout on lightly exploited wild trout populations. Special angling regulations restricted legal harvest to a small proportion of the wild trout population in the St. Joe River, and few people fished Big Springs Creek during the study.

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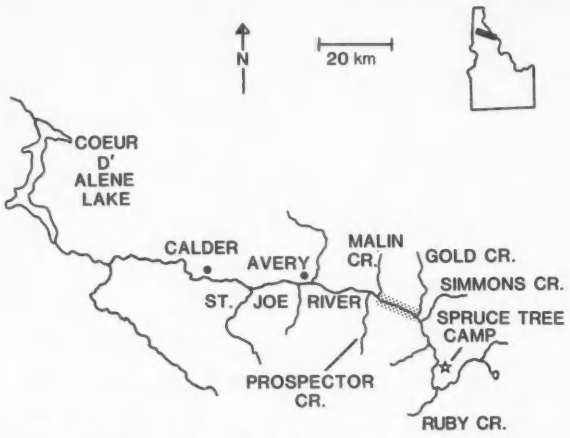

FIG. 1. St. Joe River, Idaho, and location of study zone (stippled).

Study Streams

St. Joe River

The St. Joe River, a relatively infertile stream, originates in the Bitterroot Mountains along the Idaho–Montana border and drains a 3900-km² watershed into Lake Coeur d'Alene (Fig. 1). The river above Avery was relatively pristine despite a road along the river up to Spruce Tree Camp and logging in several of the tributary watersheds. Elevation in the study zone ranged from 870 to 1000 m and the gradient averaged 7.3 $m \cdot km^{-1}$ (Johnson 1977). Total alkalinity in upper river tributaries (Gold Creek, Simmons Creek) ranged from 33 to 42 ppm and total dissolved solids range from 40 to 50 ppm (Oien 1957; Bachmann 1958). In 1979, stream discharge at Calder ranged from 4 $m^3 \cdot s^{-1}$ in November to 569 $m^3 \cdot s^{-1}$ in May; discharge during the summer study period was 13–18 $m^3 \cdot s^{-1}$.

Cutthroat trout in the section of St. Joe River we studied maintain a restricted home range (often a single pool) during July and August, begin a downstream migration to wintering areas in the lower portion of the river in late August or September, and return to upstream summer rearing areas in May and June (Johnson 1977). In addition to cutthroat trout, mountain whitefish (*Prosopium williamsoni*), northern squawfish (*Ptychochielus oregonensis*), bull trout (*Salvelinus confluentus*), and sculpin (*Cottus* sp.) were present in the study area. Schools of kokanee (*Oncorhynchus nerka*) from Lake Coeur d'Alene were observed in some sections. Rainbow trout stocked annually in the river downstream from Prospector Creek (about 2 km downstream from study area) were rare in the study zone before our experimental stocking.

Special angling regulations (three fish over 33 cm, no live bait) were imposed on the St. Joe River upstream from Prospector Creek in 1971 to reduce the mortality rate on the native cutthroat trout (Rankel 1971; Johnson and Bjornn 1978). Hatchery trout were not stocked in the special regulation area after 1970. The cutthroat trout population responded quickly to the reduced mortality rate with a four- to fivefold increase in abundance in 5 yr.

Big Springs Creek

Big Springs Creek, a productive tributary of the Lemhi River in central Idaho, originates from springs 2 km from Leadore,

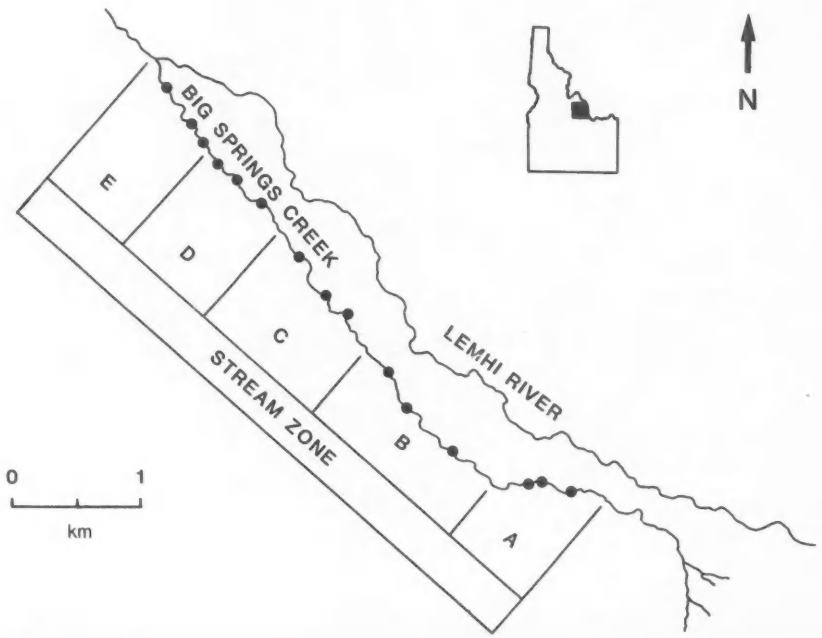

FIG. 2. Big Springs Creek, Idaho, and location of stream zones and sample sections, listed consecutively from the upstream end.

TABLE 1. Weekly counts of small (<15 cm total length), medium (15–30 cm), and combined sizes of cutthroat trout in stocked and unstocked sections, St. Joe River, July and August 1979.

	Week after initial, unstocked				Week after stocking 50 trout				Week after stocking 150 trout				Week after stocking 500 trout			
Transect	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
								Small								
1	11	9	10	2	6	3	1	1	6	4	3	0[a]	5[a]	2[a]	7[a]	3[a]
2	10	9	7	4	5	3	7	3	0[a]	0[a]	1[a]	1[a]	—	—	—	—
3	20	17	15	13	14	10	5	10	8	8	3	2	—	—	—	—
4	8	10	10	18	12	6	7	3	20	14	13	10	—	—	—	—
5	10	10	5	3	14	18	16	16	9	4	2	4	—	—	—	—
6	14	18	13	16	9	11	7	10	18	17	22	25	—	—	—	—
7	19	26	16	19	36	35	18	23	12	16	7	14	—	—	—	—
								Medium								
1	28	29	28	31	12	9	13	22	11	16	18	14	38[a]	29[a]	33[a]	23[a]
2	15	19	20	24	24	25	23	19	15	12	19	13	—	—	—	—
3	21	25	25	23	27	30	27	28	32	31	28	25	—	—	—	—
4	20	25	22	25	26	33	24	29	36	44	43	47	—	—	—	—
5	10	8	12	14	29	33	34	39	19	16	19	16	—	—	—	—
6	26	24	29	47	27	27	29	35	43	52	61	62	—	—	—	—
7	50	41	49	48	49	13	38	33	19	35	25	42	—	—	—	—
								Combined sizes								
1	47	42	39	36	21	14	15	26	21	21	22	15	46[a]	33[a]	41[a]	27[a]
2	29	31	30	29	35	31	32	23	19	16	21	15	—	—	—	—
3	44	43	42	36	44	40	37	39	46	40	35	29	—	—	—	—
4	30	36	32	43	41	40	33	33	58	62	61	58	—	—	—	—
5	22	19	18	18	45	54	53	57	30	21	21	21	—	—	—	—
6	41	43	42	63	38	41	39	46	64	72	90	89	—	—	—	—
7	70	68	65	67	87	50	57	57	32	55	36	59	—	—	—	—

[a]Excluded from statistical analysis.

Idaho (Fig. 2). The stream meanders 8 km through irrigated pastures in a high-elevation alluvial flood plain (1820 m above mean sea level) between the Beaverhead and Lemhi mountain ranges. Total alkalinity was 136 ppm and total hardness 164 ppm, both as $CaCO_3$, pH was 8.1, and total dissolved solids were 298 $mg \cdot L^{-1}$ (Bjornn 1966). Groundwater springs maintain a relatively constant discharge (1.0 $m^3 \cdot s^{-1}$) except for surface water runoff from snowmelt when snow accumulations are unusually high, as occurred in June 1982.

Naturally reproducing rainbow trout, which originated from steelhead trout (*Salmo gairdneri*) or hatchery rainbow trout stocked prior to 1962, were the dominant salmonid in Big Springs Creek during this study (1979–82). Steelhead trout and spring and summer chinook salmon (*Oncorhynchus tshawytscha*) were abundant in the Lemhi River and tributaries prior to the construction of a hydroelectric diversion dam near the mouth of the river in 1897 and development of numerous irrigation diversions (Bjornn 1966). Summer chinook salmon were eliminated from the drainage and steelhead were reduced to low numbers by the dams. Spring chinook salmon continued to be abundant because they entered the river during spring runoff when flows in the river exceeded diversion demands. During our study, chinook salmon were not allowed to spawn in Big Springs Creek. A program to reestablish steelhead trout in the Lemhi River drainage began in 1962 with the release of steelhead trout fry into Big Springs Creek each year through 1974 (Bjornn 1978; Horner 1978). During the years steelhead were stocked, they were the most abundant salmonid in Big Springs Creek. When stocking of steelhead fry ceased during 1975–80, the nonmigratory rainbow trout once again became the dominant species in the creek. Stocking of steelhead trout fry in Big Springs Creek was resumed in 1981, but we believe that the presence of age 0 steelhead in 1981 and age 1 fish in 1982 had little effect on our study because we were concentrating on older, larger fish. Mountain whitefish were common throughout Big Springs Creek, brook trout (*Salvelinus fontinalis*) and shorthead sculpin (*Cottus confusus*) were common in the upper reaches, and longnose dace (*Rhinichthys cataractae*) were common in the lower portion. Small numbers of cutthroat trout, juvenile chinook salmon, and bull trout were captured during the study.

Response of Cutthroat Trout in St. Joe River

Procedures

Changes in abundance and behavior of cutthroat trout in response to stocked catchable-size rainbow trout during summer were assessed by counting all fish and observing behavior in selected sections of the St. Joe River during July and August 1979. We selected 22 sections consisting of a pool, run, or reach of pocket water, which together made up 8% of the length of the study zone. Seven of the sections were long-established sections in which fish had been counted regularly since 1969

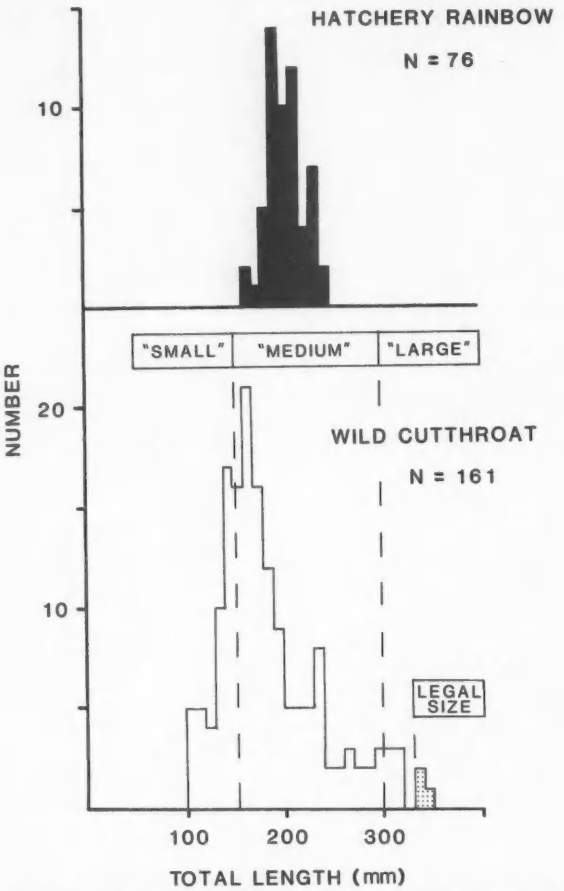

FIG. 3. Length distributions of wild cutthroat trout and stocked hatchery rainbow trout caught by angling from the study section of the St. Joe River, August 1979.

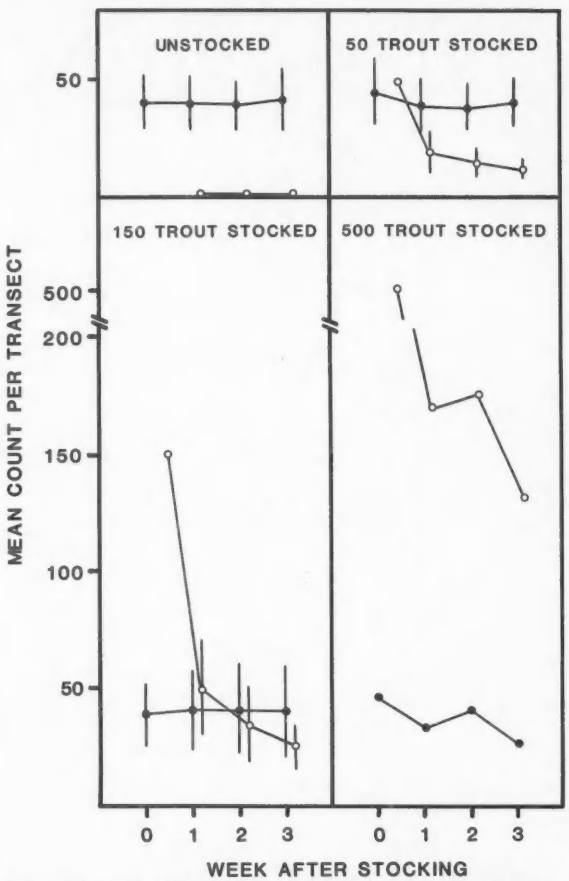

FIG. 4. Mean count of wild cutthroat trout (solid circles) and hatchery rainbow trout (open circles) in stocked and unstocked sections, St. Joe River, August 1979. Vertical lines represent ±2 SE.

(T. C. Bjornn, unpubl. data) and they served as control sections in which no hatchery trout were stocked. In 14 of the sections (average length 106 m, range 73–197 m), hatchery trout were stocked in early August at the rate of either 50 or 150 fish per section (seven of each selected randomly). One section (146 m in length) was stocked with 500 hatchery trout to serve as a behavioral observation section. Although we did not select the control (unstocked) sections randomly, they were interspersed among sections stocked with hatchery trout. A one-way classification for the main effects with repeated measures on weeks was the model used to determine effects of stocking hatchery trout on cutthroat trout abundance. Two-tailed statistical tests were conducted at the 5% level of significance. We used Duncan's multiple range test to separate mean changes in abundance by stocking level and week (Ott 1977). Standard errors for treatment means were based on mean square errors for main effects.

Cutthroat and rainbow trout in three size groups (<15, 15–30, >30 cm) were counted weekly from 29 July through 21 August 1979. Number, size group, and location of fish were recorded on a plexiglass slate by a diver with snorkeling gear drifting slowly through each transect. All counts were conducted during optimal daylight (0900–1600) and underwater visibility exceeded 5 m. Change in abundance in the sections was calculated for small (<15 cm), medium 15–30 cm), and all size groups combined of cutthroat trout and for hatchery rainbow trout as

$$\log_e (\text{count week } i/\text{initial count})$$

where i = 1, 2, and 3 wk after stocking.

We observed behavior of wild cutthroat trout in sections with and without hatchery rainbow trout. After carefully entering the water and remaining still for 5 min, we recorded sequences of agonistic and foraging behavior on an underwater slate in abbreviated narrative form. We combined seven possible types of agonistic behavior (Hartman 1965; Hahn 1977) into three classes based on degree of agressiveness. The most aggressive acts included a "nip" when one fish apparently bit or struck another with its jaws, a "drive" when a deliberate swimming motion was directed at another fish which fled, or a "charge" if the second fish did not leave. When a nipping motion did not contact the other fish, the act was called a threat-nip, which was considered comparable in aggressiveness with lateral and frontal display. "Intention" movements were more subtly defined aggressive movements and included short lunges, quick turns of the head, and other slight movements made with fins

TABLE 2. Mean weekly change in counts per section of small (<15 cm), medium (15–30 cm), and all sizes of cutthroat trout relative to initial counts (log$_e$ (count week *i*/initial count)) in stocked and unstocked sections, St. Joe River, August 1979.

Weekly interval	Section: Not stocked	50 trout stocked	150 trout stocked
	Small		
0–1	0.04	−0.26	−0.22
0–2	−0.21	−0.55	−0.82
0–3	−0.47	−0.60	−0.48
Mean ± 2 SE	−0.21 ± 0.40	−0.47 ± 0.40	−0.45 ± 0.40
	Medium		
0–1	0.02	−0.16	0.14
0–2	0.12	−0.01	0.25
0–3	0.25	0.10	0.16
Mean ± 2 SE	0.13 ± 0.18	0.02 ± 0.18	0.18 ± 0.18
	Combined sizes		
0–1	−0.05	−0.13	−0.03
0–2	−0.06	−0.15	0.00
0–3	0.01	−0.08	−0.07
Mean ± 2 SE	−0.02 ± 0.17	−0.12 ± 0.17	−0.01 ± 0.17

extended toward the other fish. Winners and losers of aggressive encounters were noted. We estimated the length of each cutthroat trout to the nearest 25 mm but did not differentiate sizes for hatchery rainbow trout.

Results

Abundance of cutthroat trout

Prior to the stocking of hatchery rainbow trout (week 0 in Table 1), sections to be left unstocked and those to be stocked contained similar numbers and sizes of cutthroat trout. Thirty percent of the fish were in the small (<15 cm total length), 63% medium (15–30 cm), and 7% large (>30 cm) size groups. Cutthroat trout caught by angling had a size distribution of 27% small, 67% medium, and 6% large (Fig. 3), which was similar to that seen snorkeling. Small cutthroat trout were comparatively scarce in the transects because they live 2–3 yr in tributaries before entering the river (Johnson 1977). Snorkeling observations and conversations with anglers confirmed that legal-size fish (>33 cm) were also scarce during August 1979.

Cutthroat trout abundance remained relatively stable during the 4-wk experiment in both stocked and unstocked sections, except for a gradual decline in the single section stocked with 500 hatchery trout (Fig. 4). Although mean counts were stable, counts in some sections varied from week to week (Table 1).

Stocking levels of 50 and 150 hatchery rainbow trout per section did not significantly change abundance of small (<15 cm), medium (15–30 cm), or combined sizes of wild cutthroat trout (Table 2). A cursory inspection of weekly mean changes suggest that stocking may have temporarily decreased abundance of smaller cutthroat trout. These data were weak, however, because few small cutthroat trout were counted in many sections (Table 1); thus, small changes in counts resulted in relatively large proportional changes. In the single section stocked with 500 catchable-size trout, abundance of medium and combined sizes of cutthroat trout decreased proportionately more (−0.30 and −0.33, respectively) than in unstocked sections (0.13 and −0.02) or those stocked with lesser numbers of hatchery trout (0.18 to −0.12) (Table 2).

The size distribution of cutthroat trout shifted during August. Small cutthroat trout declined in abundance in the weekly counts ($F = 5.02$; $p = 0.01$). The major decline occurred between the second and third week of August (Table 2). Growth by small fish into the medium size group and/or a changing visual perception of length probably accounted for most of the decline in

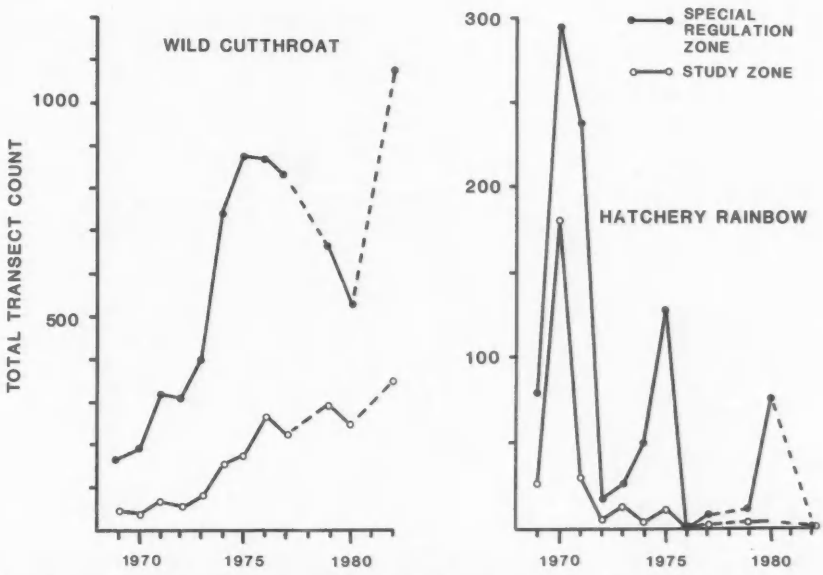

FIG. 5. Counts of wild cutthroat trout and hatchery rainbow trout during August in 21 sections in the special regulation zone and 7 sections in the present study zone, St. Joe River, 1969–82. Note difference in ordinate scale.

TABLE 3. Mean weekly change in counts per section of hatchery rainbow trout relative to initial number stocked (log$_e$ (count week i/number stocked)) in stocked transects, St. Joe River, August 1979.

Weekly interval	Section	
	50 trout stocked	150 trout stocked
0–1	−1.11	−1.23
0–2	−1.27	−1.70
0–3	−1.47	−1.89
Mean ± 2 SE	−1.29 ± 0.37	−1.61 ± 0.37

counts of small fish. The number of medium-size fish increased concomitantly ($F = 3.43$; $p = 0.04$) during August, while abundance of all sizes combined remained stable (Table 2), as indicated by logarithmic values close to zero.

Abundance of hatchery rainbow trout

Since stocking ceased in 1971, abundance of hatchery rainbow trout has declined in the special regulation zone and the 1979 study area (Fig. 5). Sporadic movement into the special regulation zone by rainbow trout stocked downstream from Prospector Creek (Fig. 1) caused some fluctuation in annual counts. Generally, hatchery rainbow trout have not intruded farther than 2 km into the special regulation zone. Few moved farther upriver than the lower boundary (Malin Creek) of the study zone.

Although 1900 hatchery rainbow trout were released into 15 sections within the study area, few were observed in the interspersed unstocked sections (Fig. 4). None were detected upstream from the study zone.

Stocking temporarily increased total trout abundance in stocked sections. Release of 50 and 150 hatchery rainbow trout approximately doubled and quadrupled total trout abundance in the stocked sections. In the section that received 500 hatchery rainbow trout, abundance increased temporarily more than 11-fold. However, hatchery rainbow trout abundance declined throughout August (Fig. 4). Three weeks after stocking, sections stocked with 50 hatchery fish contained an average of 12 rainbow trout, and those stocked with 150 had an average of 25. In the single section stocked with 500 hatchery trout, 132 remained at the end of the experiment. No more than two hatchery rainbow trout were observed in any unstocked section.

Rates of decline in abundance of hatchery rainbow trout did not differ significantly between sections stocked with 50 and 150 fish (Table 3). Abundance in the section stocked with 500 fish decreased at a slightly lesser rate (−1.15) than those with lower stocking levels.

Losses of hatchery trout were largest during the first week after stocking (Fig. 4). Significant declines ($F = 7.42$; $p < 0.01$) continued until the third week after stocking (Table 3). Similarity between logarithmic ratios of number remaining to number stocked for the periods 0–2 and 0–3 wk after stocking indicates that the rate of decline slowed.

Distribution and behavior of wild and hatchery trout

When only cutthroat trout were present in the St. Joe River study sections, they appeared to hold temporary positions from which they foraged at tails of riffles, tails of pools, and stream margins with moderate depth and velocity. In eddies and slow deep pools, cutthroat trout moved freely while foraging and did not seem to establish focal points. They avoided deep swift areas in midstream.

A size-dominance hierarchy determined feeding location and duration for individual cutthroat trout. Agonistic activity at feeding stations was apparently initiated by positioning for the opportunity to forage, not by the feeding act. Larger cutthroat trout dominated smaller trout by their presence or by intention movements. Intention movements were the most commonly observed agonistic behavior among foraging cutthroat trout (Fig. 6), especially between fish of different sizes. Aggression between cutthroat trout of similar size was expressed more overtly with more charges, drives, and nips. When a dominant fish ceased foraging and left a station, other fish adjusted position; individuals apparently had no exclusive claim to any feeding station.

In the section stocked with 500 fish, most hatchery rainbow trout segregated spatially from wild cutthroat trout (Fig. 7). Upon release, hatchery rainbow trout formed aggregations in generally deeper and swifter water in midstream than that preferred by cutthroat trout. A few small groups dispersed into

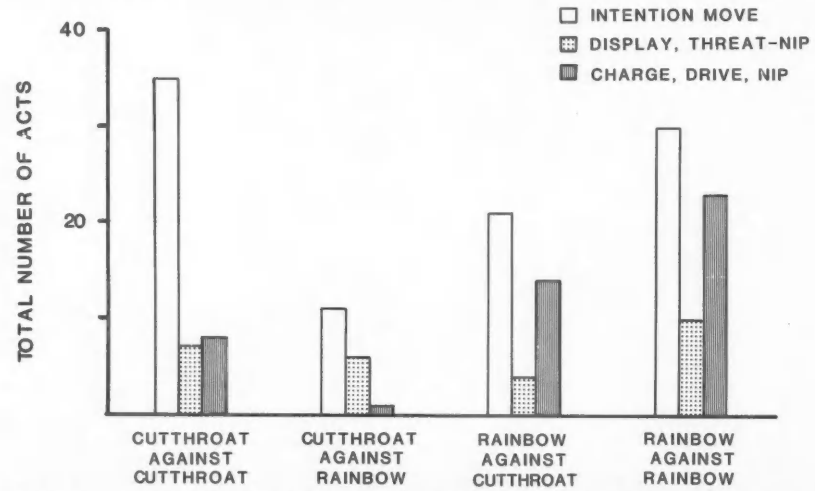

FIG. 6. Frequency of types of agonistic encounters between wild cutthroat and hatchery rainbow trout in the St. Joe River study sections, August 1979.

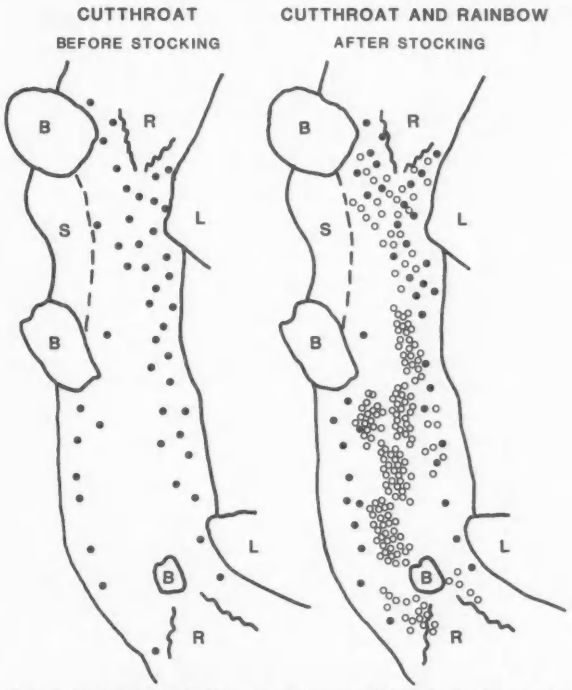

FIG. 7. Distribution of wild cutthroat trout (solid circles) before stocking and of wild cutthroat trout and hatchery rainbow trout (open circles) 2 wk after stocking 500 fish in a section of the St. Joe River, August 1979. Prominent habitat features are boundary riffles (R), emergent boulders (B), shoal (S), and rock ledges (L). Direction of flow is top to bottom.

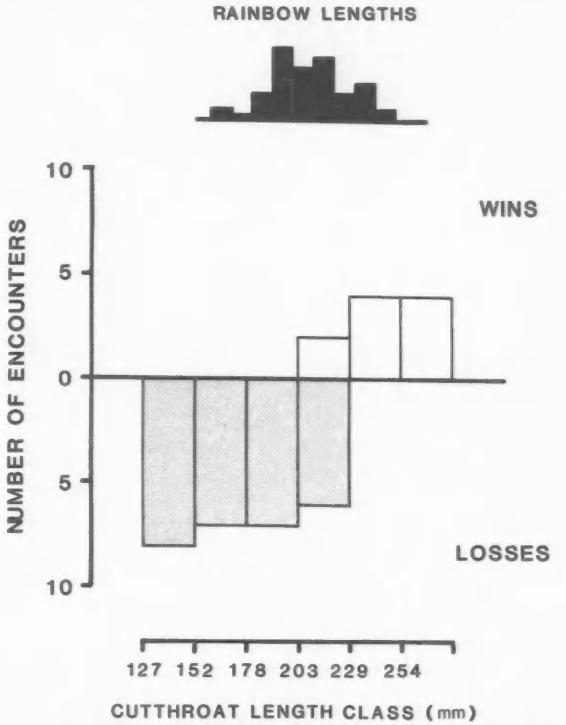

FIG. 8. Wins and losses by cutthroat trout of various sizes in aggressive encounters with hatchery rainbow trout in the St. Joe River. Length–frequency distribution of hatchery trout stocked in the sections is inset in black bars.

riffles upstream and downstream from the release point. Most hatchery trout remained in groups segregated from wild cutthroat trout throughout August (Fig. 7). Although relatively few hatchery rainbow trout used habitat similar to that used by cutthroat trout, they outnumbered wild trout in some areas of the pool.

Members of the spatially segregated hatchery trout groups were usually inactive. The groups became looser but remained intact throughout August. These aggregates had no apparent hierarchy. During infrequent feeding, several group members pursued and fought over single items of food or debris drifting past the group.

Hatchery rainbow trout that used the same habitat as cutthroat trout behaved aggressively either while in groups or as individuals. These hatchery rainbow trout displayed overt aggression toward other cutthroat or rainbow trout and charged, drove, and nipped each other proportionately more often than wild cutthroat trout (Fig. 6). A feeding attempt by either cutthroat or rainbow trout near other hatchery trout precipitated aggressive behavior by hatchery trout. Acting individually, a few hatchery rainbow trout were able to establish and defend feeding stations. Behavior of these individuals appeared similar to that of wild cutthroat trout. Wild cutthroat and hatchery rainbow trout apparently reacted to each other as they did to conspecifics (Fig. 6). Although the number of observations was small, cutthroat trout used more intention movements whereas rainbow trout tended to act more overtly.

Size was a primary factor in determining the winner of agonistic encounters between wild cutthroat and hatchery rainbow trout (Fig. 8). Cutthroat trout estimated to be smaller than 20 cm never won an encounter with the larger hatchery trout, whereas cutthroat trout estimated to be longer than 23 cm never lost. Cutthroat trout similar in size to hatchery trout won some of the encounters. When a cutthroat trout lost an encounter to a large wild or hatchery trout, it moved to another nearby feeding station.

Response of Wild Rainbow Trout in Big Springs Creek

Procedures

The responses of wild rainbow trout to hatchery trout were assessed in study sections of Big Springs Creek by estimating the abundance of wild trout and tagging the larger fish each June of 1979–81 before stocking and then estimating abundance and recapturing tagged fish in September–October and the following June of 1980–82. Abundance, movements, survival rates, growth, and condition factors of wild trout were the responses monitored.

A randomized block experimental design was chosen to account for environmental differences along the length of Big Springs Creek. We established five stream zones (blocks) and within each zone selected three similar sections, each containing at least three pools and bounded by riffles (Fig. 2). Sections averaged 135 m in length (range 93–185 m); the combined

TABLE 4. Number of hatchery rainbow trout stocked and estimated abundance and 95% confidence intervals based on subsequent fall and spring electrofishing samples, Big Springs Creek, 1979–82.

Stream zone	Section	Area (m^2)	1979 Number stocked	1979 Fall estimate	1980 Spring estimate	1980 Number stocked	1980 Fall estimate	1981 Spring estimate	1981 Number stocked	1981 Fall estimate	1982 spring estimate
A	1	957	0	1	0	0	4 ± 0	0	400	99 ± 1	0
	2	979	100	12 ± 0	0	100	22 ± 1	0	0	6 ± 2	0
	3	744	50	28 ± 0	0	50	14 ± 0	—	—	—	1
B	4	1304	0	0	0	0	1	0	400	40 ± 1	1
	5	1548	100	11 ± 1	0	100	11 ± 1	0	0	6 ± 0	0
	6	1368	50	2	0	50	11 ± 1	—	—	—	0
C	7	1770	100[a]	19 ± 0	1	400[a]	96 ± 1	0	0	6 ± 0	0
	8	1348	0[a]	6 ± 0	0	0[a]	14 ± 2	0	400	59 ± 1	2
	9	1310	50[a]	15 ± 0	2	200[a]	55 ± 4	—	—	—	0
D	10	1075	0	2	0	0	6 ± 2	0	400	159 ± 1	—[b]
	11	1186	100	32 ± 0	1	100	33 ± 0	3	0	9 ± 1	0
	12	1315	50	19 ± 1	0	50	20 ± 0	—	—	—	0
E	13	1509	50	10 ± 1	0	50	15 ± 4	—	—	—	2
	14	1159	0	6 ± 2	0	0	6 ± 2	0	400	107 ± 1	4
	15	1648	100	9 ± 0	0	100	17 ± 1	0	0	5 ± 0	0
Streamwide			750	300[c]	8[c]	1200	500[c]	7[c]	2000	700[c]	14[c]

[a]Pilot study, excluded from 1979–80 experiment.

[b]Not sampled due to high flow.

[c]Sum of estimated abundance of fish remaining in stocked sections and proportionate expansion by stream zone of numbers that dispersed into other sections.

length of the 15 sections made up 20% of the length of Big Springs Creek.

In the experiment conducted in 1979–80, sections within each stream zone were randomly assigned one of three treatments: left unstocked, or stocked by a single release of 50 or 100 catchable-size rainbow trout (3–10 fish·100^{-2}; Table 4). Only four of the five stream zones were used as blocks in the 1979–80 experiment. Zone C was used for pilot studies of stocking regimes.

In 1981 we altered the experimental design. All five stream zones were used as blocks, each containing a single stocked and unstocked section (Table 4). Stocked sections in 1981 each received 400 hatchery trout (30–40 fish·$100m^{-2}$) released in lots of 100 at 2-wk intervals in July and August.

Catchable-size rainbow trout (mean length and weight about 24 cm and 150 g) for the experiments were provided from the Idaho Department of Fish and Game's Mackay Fish Hatchery. All hatchery rainbow trout were tagged with serially numbered Monel metal jaw-tags; individual tag numbers and total lengths (millimetres) were recorded.

Differences in responses were tested by two-tailed statistical tests at the 5% level of significance. We used Duncan's multiple range test to separate treatment, block, and yearly (1979 and 1980) means (Ott 1977). Standard errors for treatment means were based on treatment × block mean square errors. Fisher's protected least squares difference test was used to separate means for significant interaction terms (stocking level × time).

Abundance of wild rainbow trout in the sections was estimated with a two-catch removal method (Seber and LeCren 1967). Each catch was obtained by electrofishing upstream and then downstream through a section. Stunned fish were netted and placed in plastic containers in the stream for later counting, tagging, and measurement. Abundance was estimated during the periods 10–15 June 1979, 20–24 September 1979, 20–26 June 1980, 7–11 October 1980, 20–29 June 1981, 6–10 October 1981, and 18–26 June 1982.

We tagged all wild rainbow trout longer than 16 cm with distinctively numbered No. 7 and 8 Monel metal jaw-tags each June in 1979–81. Records kept for tagged fish included tag number, total length (millimetres), and weight (grams); untagged fish were recorded by 10-mm length interval. All fish were released back into the section from which they were collected. Short-term mortality attributable to sampling was less than 1%.

Wild rainbow trout were separated into approximate age groups based on length distributions (Fig. 9), and the abundance of each such length/age class was estimated separately (Seber and LeCren 1967). Wild subyearlings in fall were designated as length-class 0 (Fig. 9). In 1981, length–class 0 included subyearling steelhead stocked earlier in the summer that could not be distinguished from wild rainbow trout. Yearling wild rainbow trout were subdivided into two length classes, IA and IB, because about 99% of the stocked rainbow trout were longer than length-class IA. Length-classes II and III corresponded approximately to the sizes of wild rainbow trout of age 2 and ages 3 and older, respectively.

Effects of stocking level on abundance of wild rainbow trout were tested on the basis of densities of subyearlings in fall and changes in abundance over summer for rainbow trout in length-classes IA and IB and II (Table 5); rainbow trout in length-class III were too sparsely represented in the sections to support valid statistical evaluation of changes in abundance.

Emigration of rainbow trout from Big Springs Creek was monitored at a fish weir in 1979 and 1980. A diversion dam near the mouth of Big Springs Creek diverted the stream through a rotary drum screen that guided downstream migrants into a

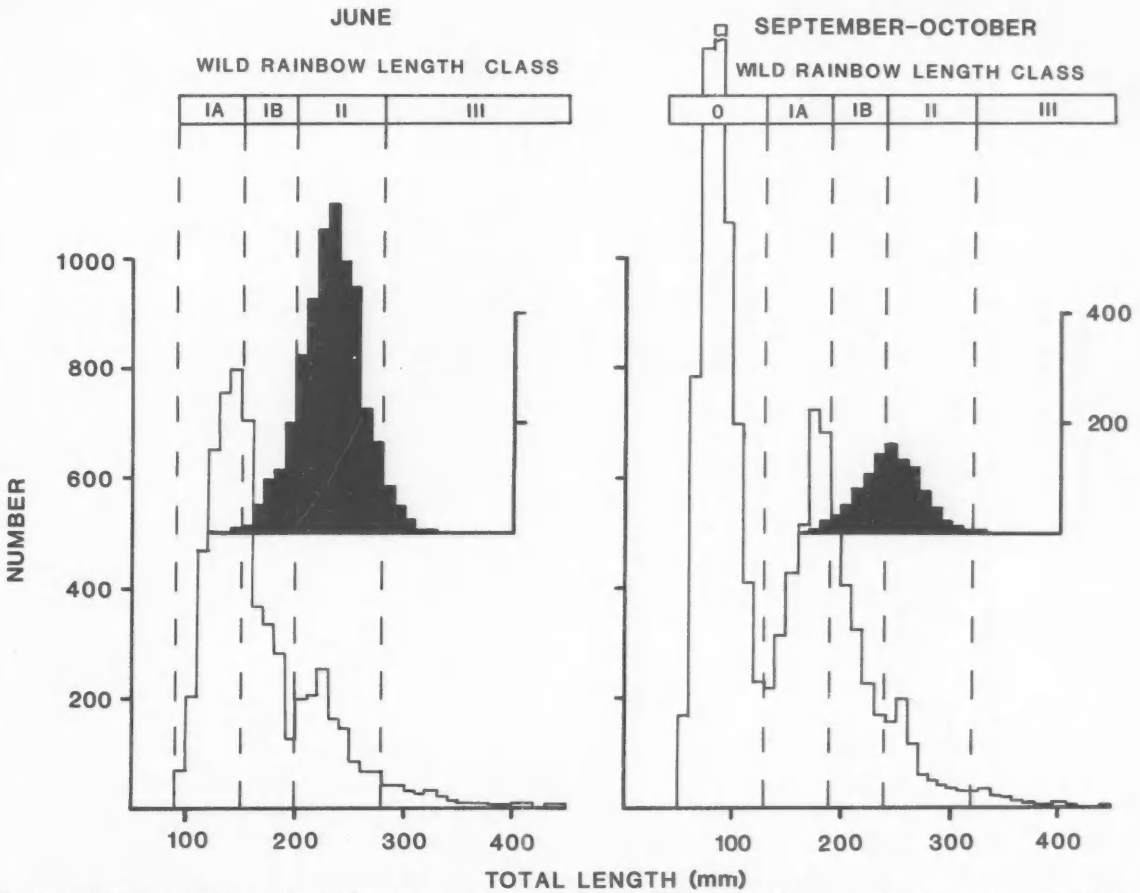

FIG. 9. Length–frequency distribution of wild rainbow trout in June and in fall and of hatchery rainbow trout (inset) at stocking and in fall, Big Springs Creek, 1979–81.

trap (Bjornn 1978). The weir was operated from 22 March to 18 November 1979 and from 6 May to 17 November 1980.

Each known survivor (a tagged fish recaptured during or after a given sampling period) was classified as either a summer nonmigrant or migrant on the basis of recapture location during the fall population estimate. By definition, nonmigrants were fish recaptured in fall in the same section where they were initially captured and tagged. All other known survivors were classed as migrants.

The degree of exposure of migrants to the experimental conditions (competition with hatchery trout) is unknown. The question of whether nonmigrants remained within section boundaries was tested by a single electrofishing survey in mid-August 1980, when about 60% of Big Springs Creek was sampled. Each section and a 100-m reach above and below each section were included in the survey.

The effects of stocking on dispersal of wild rainbow trout from the sections were tested by two ratios: numbers of migrants in fall divided by initial number and number of migrants divided by known survivors in fall (Table 5). Interpretation of both ratios in terms of dispersal would be affected by differential mortality of migrants. We used an arc-sine square root transformation (Mosteller and Youtz 1962) to stabilize variances and moderate the effects of extreme values from small samples (Cargill 1980).

Transformed instantaneous summer and annual mortality rates (Ricker 1975) of known survivors and nonmigrants from each section were also used to test for effects of stocking level. We transformed the mortality rates by adding 1.0 to the numerator (final number) and denominator (initial number) before taking natural logarithms (Table 5). Without transformation, instantaneous mortality rates for the few categories in which no fish survived would be undefined.

We estimated instantaneous growth rates (Ricker 1975) during summer for all tagged wild rainbow trout recaptured in fall. Effects of stocking on instantaneous growth rates of nonmigrants were tested for yearlings and for fish 2 yr old and older, here termed overyearlings (Table 5).

Condition factors (Everhart et al. 1975) for tagged rainbow trout were calculated (total length in millimetres and weight in grams) based on an assumption of isometric growth. Effects of stocking on end-of-summer condition factor (K) of nonmigrants were tested for yearling and overyearling rainbow trout (Table 5).

Results

Population dynamics of wild rainbow trout

Abundance of wild rainbow trout in stream zones A–E varied moderately from June 1979 through June 1982 (Table 6) and

TABLE 5. Summary of parameters estimated in stocked and unstocked sections, Big Springs Creek, 1979–80 and 1981 experiments.

Population parameter	Period	Test group
Density	Fall (Sept.–Oct.)	Subyearlings[a]
Change in abundance[b]	Summer (June–fall)	Small yearlings (length class IA)
		Large yearlings (length class IB)
		Age 2 (length class II)
Proportion of migrants to initial number[c]	Summer	Tagged yearlings and overyearlings
Proportion of migrants to known survivors[c]	Summer	Tagged yearlings and overyearlings
Instantaneous mortality rate[d]	Summer	Known survivors
		Nonmigrants
	Annual (June–June)	Known survivors
		Nonmigrants
Instantaneous growth rate	Summer	Yearling nonmigrants
		Overyearling nonmigrants
Condition factor	Fall	Yearling nonmigrants
		Overyearling nonmigrants

[a]Resident rainbow trout in 1979–80; resident rainbow trout and introduced steelhead trout in 1981.

[b]Log$_e$ (fall abundance/June abundance).

[c]Statistical analyses performed on transformed (arc-sine square root) proportions.

[d]Transformed by adding 1.0 to final and initial numbers before taking natural logarithms.

was similar to comparable estimates in 1975 for the same reach of stream (Horner 1978). The increase in abundance of subyearlings (age 0 fish) in the fall of 1981 was the result of the release of steelhead fry early that summer.

Movements of wild rainbow trout in Big Springs Creek were of several types. Adults moved upstream in March to spawn, primarily to stream zones A–B and in the headwaters. After spawning, fish that had entered Big Springs Creek in March to spawn returned to the Lemhi River. Subyearlings and some yearlings gradually dispersed downstream, with some emigrating from Big Springs Creek during fall, winter, and spring. Most yearling and older rainbow trout appeared to have a restricted home range (Petrosky 1984).

Many tagged rainbow trout were found in the same section in fall as in the preceding June. These nonmigrants recaptured in fall made up 84% of all recaptured tagged wild rainbow trout (3-yr weighted average; Table 6), 74% of the known survivors, and 60% of the extrapolated number of tagged fish at large. Tagged wild trout also tended to be in the same stream sections in successive June sampling periods (73–91% nonmigrants; Table 6).

During the August 1980 electrofishing survey to assess possible summer movements, we found that most wild rainbow trout classed as nonmigrants on the basis of June and October sampling did not move far from their home section during summer. Of 211 tagged wild trout classified as nonmigrants in 1980 on the basis of June and October locations, 48% were recaptured within their home section, 5% were found outside the section occupied in June, and 46% were not collected during the single upstream pass. Only two fish, classified as nonmigrants, were recaptured in mid-August more than 100 m from their locations in June; these had moved 280 and 600 m from their initial sections before returning by October.

Mortality of wild rainbow trout was apparently relatively high if the approximately 87% annual loss of tagged wild trout is representative of all wild trout. Mortality of yearling and older wild trout was lower in 1979 than in 1980 or 1981 (Table 6).

Growth of tagged wild trout was faster in 1980 and 1981 than in 1979 (Table 6). The trend of increasing growth rates from 1979 to 1981, if real, was associated with several factors: a decrease in abundance of older rainbow trout, a decrease in initial condition factors each June (Table 6), and perhaps variations in unmeasured environmental conditions. In 1979, age 2 and older rainbow trout did not gain weight between June and September. Condition factors in June varied by age group and year, but were similar each fall (Table 6).

Response of wild rainbow trout to stocking

Stocking of hatchery rainbow trout did not affect fall densities of wild subyearlings in 1979 and 1980, but may have reduced the density of subyearlings in 1981, the year steelhead fry were stocked in spring (Table 7). In all three years, densities of wild subyearlings in unstocked sections did not differ significantly from those stocked with hatchery rainbow trout (Table 7). In 1981, densities of wild rainbow trout and introduced steelhead subyearlings were lower in sections of zones A–D stocked with 400 catchable-size trout than in unstocked sections, but the difference was not significant at the 0.05 level ($F = 5.21$; $p = 0.08$). If the stocking of 400 catchable-size rainbow trout reduced the density of subyearlings in stocked sections in 1981, the density (0.28 fish·m^{-2}) still exceeded the overall density in 1979 and 1980 (0.23 fish·m^{-2}).

Subyearling trout were significantly less abundant in downstream sections (D and E) than in upstream sections (A and B) in 1979 and 1980 (when only wild rainbow trout were present: $F = 92.91$; $p < 0.01$) and in 1981 (when both wild rainbow trout and introduced steelhead were present: $F = 48.93$; $p < 0.01$) (Table 7). Because of the strong density trend from the upper to the lower end of the stream, the blocked experimental design was 20 times more efficient than an unblocked design would have been for detecting the potential effects of stocking.

Stocking of hatchery rainbow trout did not cause wild rainbow trout to leave Big Springs Creek in either 1979 or 1980. Only 33 of 2039 tagged wild rainbow trout were captured in the weir during the two years. About half (17) were caught in May 1980, before hatchery trout were stocked for that year. Of the rest, five, four, and seven wild trout originated from sections that were not stocked, stocked with 50 hatchery trout, and stocked with 100 hatchery trout, respectively.

Hatchery rainbow trout did not displace tagged wild rainbow trout from the sections. If simple displacement had occurred, without an increase in mortality, the proportions of wild fish classed as migrants (based on initial number or known survivors) would have been significantly different between stocked and unstocked sections, but such was not the case (Table 8). If some wild trout migrated from sections and later died from interactions with stocked trout, displacement might not have been detected.

Summer mortality, but not annual mortality, of fish present before stocking was higher in 1981 in the sections with the highest stocking level (400 hatchery trout) than in unstocked

TABLE 6. Estimated population parameters (±2 SE) for wild rainbow trout, Big Springs Creek, 1979–81.

Population parameter	Period	Group	1979	1980	1981
Abundance	June[a]	Age 1	5933 ± 539	6902 ± 673	5521 ± 644
		≥Age 2	2024 ± 312	1794 ± 283	1379 ± 249
	Fall	Age 0	13 769 ± 1084	11 985 ± 2438	22 929 ± 3321[b]
		Age 1	7506 ± 876	4345 ± 456	6225 ± 1427
		≥Age 2	1170 ± 193	926 ± 247	605 ± 142
Emigration from Big Springs Creek	May–November	Age 0	619	1492	—
		Age 1	499	927	—
		≥Age 2	784	710	—
Proportion of nonmigrants[c]	Summer	Tagged yearlings	0.83	0.84	0.92
		Tagged overyearlings	0.83	0.81	0.88
	Annual	Tagged yearlings	0.73	0.74	0.91
		Tagged overyearlings	0.91	0.80	0.75
Instantaneous mortality rate	Summer	Tagged yearlings	1.79	3.19	2.34[d]
		Tagged overyearlings	1.76	1.91	2.19[d]
	Annual	Tagged yearlings	1.93	2.64	2.71
		Tagged overyearlings	1.83	2.05	2.08
Instantaneous growth rate	Summer	Tagged yearlings	0.29 ± 0.14	0.95 ± 0.20	1.12 ± 0.23
		Tagged overyearlings	0.01 ± 0.10	0.47 ± 0.11	0.66 ± 0.11
Condition factor	June	Tagged yearlings	1.26 ± 0.01	1.18 ± 0.02	1.09 ± 0.02
		Tagged overyearlings	1.18 ± 0.01	1.08 ± 0.02	1.03 ± 0.01
	Fall	Tagged yearlings	1.00 ± 0.02	1.01 ± 0.02	1.04 ± 0.04
		Tagged overyearlings	1.01 ± 0.01	1.04 ± 0.02	1.04 ± 0.02

[a]Estimated abundance in June 1982 was 3809 ± 526 (age 1) and 1018 ± 141 (age 2).
[b]Includes stocked subyearling steelhead trout, which could not be distinguished from subyearling rainbow trout.
[c]Number recaptured from initial section/total number recaptured.
[d]Excludes stocked sections where mortality rate was influenced.

TABLE 7. Fall density (number·m^{-2}) of wild rainbow trout (1979 and 1980) and wild rainbow and introduced steelhead trout (1981) subyearlings in relation to various stocking treatments in Big Springs Creek.

		Treatment			
Year	Stream zone	Not stocked	50 trout stocked	100 trout stocked	400 trout stocked
1979	A	0.554	0.732	0.563	—
	B	0.301	0.332	0.369	—
	D	0.020	0.027	0.033	—
	E	0.011	0.019	0.008	—
1980	A	0.657	0.366	0.414	—
	B	0.218	0.352	0.286	—
	D	0.041	0.039	0.030	—
	E	0.010	0.019	0.005	—
	Mean ± 2 SE	0.23 ± 0.05	0.24 ± 0.05	0.21 ± 0.05	—
1981	A	0.928	—	—	0.705
	B	0.593	—	—	0.431
	C	0.216	—	—	0.150
	D	0.114	—	—	0.077
	E	0.028	—	—	0.035
	Mean ± 2 SE	0.38 ± 0.06	—	—	0.28 ± 0.06

sections (Table 9). Releases of 100 catchable-size trout per section four times during the summer in 1981 increased summer mortality of both the known survivors ($F = 16.35$; $p = 0.03$) and nonmigrants ($F = 12.50$; $p = 0.04$). As judged by mortality rates of known survivors in 1981, 25% fewer wild rainbow trout would be expected in fall with stocking of 400 trout per section (Fig. 10), but no difference would be expected after a full year. Annual mortality (from the time of tagging to the following June) was unaffected by stocking hatchery trout at any level.

The presence of seasonal mortality effects, and lack of annual effects, may either reflect compensation in the wild rainbow trout population over winter or be an artifact of small sample size. Few tagged wild rainbow trout (average 3.4 known survivors and 1.8 nonmigrants per section in 1981) survived the year from either stocked or unstocked sections (Table 9).

TABLE 8. Proportion of wild rainbow trout in fall that had left their initial section during summer (migrants) compared with initial number tagged in June (in parentheses) and with number of known survivors in fall (in parentheses) in stocked and unstocked sections, Big Springs Creek, 1979–81.

Year	Stream zone	Treatment: Not stocked	50 trout stocked	100 trout stocked	400 trout stocked
		Migrants: initial number			
1979	A	0.16 (105)	0.19 (64)	0.11 (75)	—
	B	0.07 (58)	0.06 (63)	0.13 (104)	—
	D	0.04 (93)	0.08 (77)	0.12 (57)	—
	E	0.16 (44)	0.12 (73)	0.06 (65)	—
1980	A	0.16 (68)	0.22 (69)	0.13 (65)	—
	B	0.08 (73)	0.07 (55)	0.10 (120)	—
	D	0.06 (48)	0.11 (37)	0.15 (39)	—
	E	0.19 (26)	0.12 (26)	0.11 (54)	—
	Weighted mean[a]	0.10	0.12	0.11	—
1981	A	0.04 (47)	—	—	0.09 (53)
	B	0.09 (55)	—	—	0.09 (32)
	C	0.13 (79)	—	—	0.11 (44)
	D	0.08 (40)	—	—	0.04 (51)
	E	0.15 (46)	—	—	0 (37)
	Weighted mean[a]	0.10	—	—	0.07
		Migrants: known survivors			
1979	A	0.29 (58)	0.30 (40)	0.14 (58)	—
	B	0.13 (30)	0.13 (32)	0.23 (56)	—
	D	0.07 (58)	0.19 (35)	0.17 (36)	—
	E	0.64 (11)	0.38 (24)	0.23 (17)	—
1980	A	0.52 (21)	0.60 (25)	0.33 (27)	—
	B	0.29 (21)	0.22 (18)	0.24 (50)	—
	D	0.12 (25)	0.33 (122)	0.33 (18)	—
	E	0.50 (10)	0.38 (8)	0.33 (18)	—
	Weighted mean[a]	0.21	0.29	0.23	—
1981	A	0.09 (22)	—	—	0.28 (18)
	B	0.23 (22)	—	—	0.33 (9)
	C	0.35 (29)	—	—	0.31 (16)
	D	0.17 (18)	—	—	0.31 (16)
	E	0.32 (22)	—	—	0 (11)
	Weighted mean[a]	0.23	—	—	0.21

[a]Statistical analyses performed on transformed (arc-sine square root) proportions.

Even though tagged fish in the stocked sections suffered additional mortality in summer 1981, fall abundance (tagged and untagged wild fish, length-classes IA, IB, and II) did not change differentially between stocked and unstocked sites (Table 10). Untagged fish that immigrated into stocked sections made up for losses of tagged fish.

Mortality of wild rainbow trout differed among stream zones. Fish present in June in stream zone E died at a significantly faster rate ($F = 5.55$; $p = 0.04$) than in other zones in 1979–80, but not in 1981 (Table 9). The disappearance rate of nonmigrants did not differ significantly between stream zones in either the 1979–80 or 1981 experiments.

Stocking of hatchery trout had no measurable effect on growth rates of tagged wild rainbow trout that remained in their initial sections (Table 11), although variable growth among individuals could have masked small differences. In 1981, the mean instantaneous growth rate of wild yearling and older trout was smaller in stocked than in unstocked sections, but the difference was not significant.

Growth of older wild rainbow trout in 1981 was significantly faster ($F = 7.22$; $p < 0.01$) in the middle zones (B, C, and D) of the stream than in the upstream or downstream zones (A or E). Growth of yearling rainbow trout in 1981 and yearling and older rainbow trout in 1979–80 did not differ significantly between stream zones.

Interactions with hatchery rainbow trout throughout summer did not affect condition factor of surviving wild rainbow trout in the fall. Condition factors in fall for tagged yearling and older nonmigrants in unstocked sections did not differ significantly from those of fish in sections stocked with 50, 100, or 400 hatchery trout (Table 12).

Condition factors of nonmigrant rainbow trout varied occasionally with location in Big Spring Creek. Condition factors were similar for fish in all stream zones in 1979–80, but in 1981 the condition factors of yearling nonmigrant trout were lower in stream zone E ($F = 2.53$; $p = 0.051$) than in other zones.

Discussion

There was only limited evidence that would lead to rejection of our overall hypothesis of no detrimental effects on wild cutthroat and rainbow trout from stocking catchable-size hatchery rainbow trout in the infertile or fertile streams we studied. We anticipated that wild trout might be displaced from their normal feeding stations through one-on-one agonistic behavior with hatchery trout, or because the wild trout might not be willing to put up with the large numbers of hatchery trout suddenly thrust into their home range. In the St. Joe River, a few cases of wild trout displacement to a different feeding station within the same section were observed, but wild trout abundance did not decline in response to stocking rates of 50 or 150 trout per section (Fig. 4). Wild trout abundance declined slightly in the single pool stocked with 500 hatchery trout, but that treatment was unreplicated and thus only limited credence can be attached to that observation. In Big Springs Creek, adding hatchery trout to sections of the stream did not reduce densities of wild fish in the fall and did not cause wild trout to leave the stream or the section they were in before stocking.

Another anticipated effect of stocking hatchery trout would be an increase in mortality rates if wild trout were displaced into unsuitable environments. Consistent with little evidence of displacement, summer and annual mortality rates of tagged wild rainbow trout in Big Springs Creek were not increased by the stocking of hatchery trout, except in summer 1981 when 400 trout were stocked per section (Table 9). Although the mean mortality rate for tagged wild trout was higher in stocked versus unstocked sections during the June–October period of 1981, the stocked sections did not have proportionately fewer yearling and older trout in fall than unstocked sections (Table 10), presumably because untagged wild trout moved in and replaced any tagged trout that left or died.

Decreases in growth rate or condition factor were also potential adverse effects of stocking trout if the hatchery fish survived, fed actively, and reduced the amount of food available to wild trout. In Big Springs Creek where growth of tagged fish was monitored, the differences in growth rates or condition fac-

TABLE 9. Transformed summer and annual instantaneous mortality rates for wild rainbow trout that were known to be survivors in fall and the following June and those that did not migrate from initial sections during summer (numbers of known survivors and nonmigrants in parentheses) in stocked and unstocked sections, Big Springs Creek, 1979–81. Asterisks denote differences from unstocked sections significant at $p < 0.05$

Period	Year	Stream zone	Treatment: Not stocked	50 trout stocked	100 trout stocked	400 trout stocked
			Known survivors			
Summer	1979	A	2.56 (59)	2.08 (40)	1.07 (59)	—
		B	2.90 (30)	2.98 (32)	2.75 (56)	—
		D	2.10 (58)	3.36 (36)	2.46 (32)	—
		E	5.96 (11)	4.89 (24)	5.85 (17)	—
	1980	A	3.73 (21)	3.23 (25)	2.79 (27)	—
		B	3.95 (21)	3.36 (19)	2.82 (50)	—
		D	2.07 (25)	3.25 (13)	2.43 (18)	—
		E	2.93 (10)	3.58 (8)	3.46 (18)	—
		Mean ± 2 SE	3.27 ± 0.40	3.23 ± 0.40	3.07 ± 0.40	—
	1981	A	2.49 (22)	—	—	3.36 (19)
		B	3.01 (22)	—	—	4.04 (9)
		C	3.31 (29)	—	—	3.29 (16)
		D	2.78 (17)	—	—	3.78 (16)
		E	2.42 (22)			3.90 (11)
		Mean ± 2 SE	2.80 ± 0.16	—	—	3.67 ± 0.16*
Annual	1979	A	1.89 (15)	1.23 (18)	1.50 (16)	—
		B	1.88 (8)	1.96 (8)	1.36 (26)	—
		D	2.15 (10)	2.75 (4)	2.96 (2)	—
		E	2.42 (3)	2.69 (4)	2.80 (3)	—
	1980	A	2.29 (6)	2.05 (8)	2.80 (3)	—
		B	2.70 (4)	2.64 (3)	2.16 (13)	—
		D	1.69 (8)	3.64 (0)	2.08 (4)	—
		E	2.60 (1)	2.60 (1)	1.81 (3)	—
		Mean ± 2 SE	2.20 ± 0.40	2.47 ± 0.40	2.16 ± 0.40	—
	1981	A	3.18 (1)	—	—	2.87 (2)
		B	2.23 (5)	—	—	2.80 (1)
		C	2.30 (1)	—	—	1.86 (6)
		D	3.02 (7)	—	—	—[a]
		E	1.90 (6)	—	—	2.54 (2)
		Mean ± 2 SE	2.53 ± 0.16	—	—	2.52 ± 0.18
			Nonmigrants			
Summer	1979	A	4.07 (42)	3.64 (28)	1.80 (50)	—
		B	3.52 (26)	3.41 (29)	4.24 (40)	—
		D	2.33 (55)	4.01 (31)	2.97 (29)	—
		E	9.90 (4)	6.13 (18)	7.32 (12)	—
	1980	A	5.99 (10)	6.03 (10)	3.89 (19)	—
		B	4.99 (15)	4.76 (12)	3.69 (38)	—
		D	2.47 (22)	4.69 (8)	2.99 (15)	—
		E	5.50 (4)	4.40 (6)	4.70 (12)	—
		Mean ± 2 SE	4.85 ± 0.57	4.64 ± 0.57	3.95 ± 0.57	—
	1981	A	2.96 (19)	—	—	4.81 (12)
		B	4.03 (16)	—	—	4.79 (7)
		C	4.69 (19)	—	—	4.76 (10)
		D	3.40 (14)	—	—	4.20 (14)
		E	3.64 (15)	—	—	4.19 (10)
		Mean ± 2 SE	3.74 ± 0.24	—	—	4.55 ± 0.24*

TABLE 9. (*Concluded*).

Period	Year	Stream zone	Treatment: Not stocked	50 trout stocked	100 trout stocked	400 trout stocked
Annual	1979	A	3.05 (4)	1.98 (8)	1.63 (9)	—
		B	2.69 (3)	2.37 (5)	1.82 (16)	—
		D	2.46 (7)	3.26 (2)	3.37 (1)	—
		E	3.11 (1)	3.21 (2)	3.50 (1)	—
	1980	A	3.54 (1)	3.56 (1)	3.50 (1)	—
		B	3.21 (2)	4.03 (0)	3.70 (2)	—
		D	2.10 (5)	3.64 (0)	2.59 (2)	—
		E	3.30 (0)	3.30 (0)	2.62 (3)	—
		Mean ± 2 SE	2.93 ± 0.57	3.17 ± 0.57	2.84 ± 0.57	—
	1981	A	3.87 (0)	—	—	3.99 (0)
		B	2.93 (2)	—	—	2.80 (0)
		C	2.59 (5)	—	—	2.20 (4)
		D	3.02 (1)	—	—	—[a]
		E	2.75 (2)	—	—	2.54 (2)
		Mean ± 2 SE	3.03 ± 0.24	—	—	2.88 ± 0.27

[a]Not sampled due to high flow.

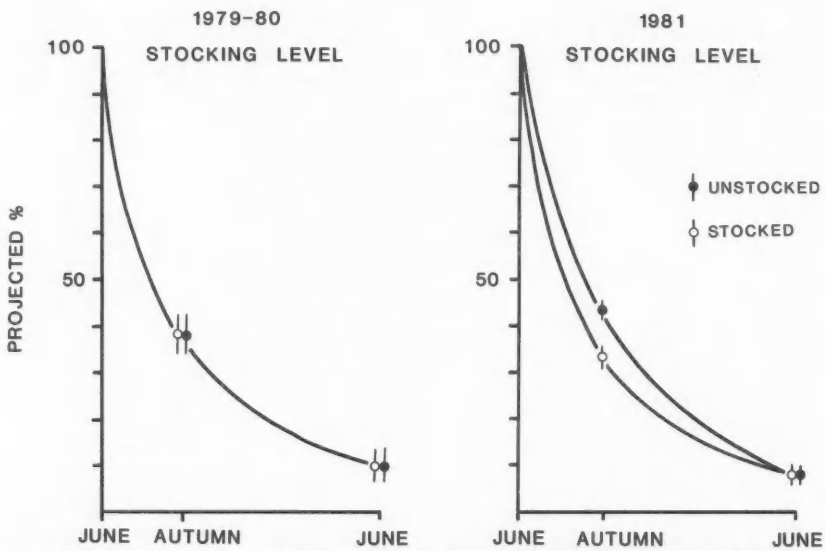

FIG. 10. Projected percentage of wild rainbow trout survivors based on summer and annual mortality rates of known survivors from stocked and unstocked sections, Big Springs Creek, 1979–80 (50 and 100 stocked trout per section) and 1981 (400 stocked trout per section) experiments. Vertical lines represent ± SE of mortality rates.

tors of wild trout in stocked versus unstocked sections were not significant (Tables 11 and 12).

Our findings of limited short-term effects of hatchery trout on wild trout in two Idaho streams would appear on first reading to conflict with findings of Vincent (1987) in two Montana streams. Vincent (1987) concluded that cessation of stocking catchable-size rainbow trout was the reason for twofold and eightfold increases in the abundance of age 2 and older brown (*Salmo trutta*) and rainbow trout, respectively, in the Madison River. In O'Dell Creek, a spring fed tributary of the Madison River, Vincent (1987) reported a 49% reduction in the abundance of age 2 and older brown trout resulting from 3 yr of stocking rainbow trout in a previously unstocked section of the stream. Age 1 wild trout were unaffected by stocking in both of the Montana streams.

Our findings are similar to Vincent's (1987) in that yearling wild trout appeared to be unaffected by the stocked trout. We differ in that we found little evidence of hatchery trout affecting age 2 and older wild trout, but Vincent (1987) concluded that stocking of hatchery trout was the primary cause of reduced numbers of the older wild trout. The populations we studied differed in that age 2 and older rainbow trout made up only 10% of the age 1 and older population in Big Springs Creek versus 36–38% of the brown trout and 44% of the rainbow trout in the Montana streams.

TABLE 10. Change in abundance (log$_e$ fall abundance/June abundance) of wild rainbow trout length-classes IA, IB, and II during summer in stocked and unstocked sections, Big Springs Creek, 1979-81. A value of 0 represents no change in abundance, 0.69 a doubling, and −0.69 a halving (from June to fall).

			Treatment			
Length class	Year	Stream zone	Not stocked	50 trout stocked	100 trout stocked	400 trout stocked
IA	1979	A	0.17	0.91	0.72	—
		B	0.17	0.80	0.95	—
		D	1.40	0.30	0.35	—
		E	0.27	0.41	0	—
	1980	A	−0.55	−0.45	−0.77	—
		B	−0.58	−0.62	−0.70	—
		D	−0.26	−0.68	−0.88	—
		E	−1.22	−0.50	−0.99	—
		Mean ± 2 SE	−0.08 ± 0.33	0.02 ± 0.33	−0.16 ± 0.33	—
	1981	A	0.15	—	—	−0.47
		B	0.22	—	—	0.09
		C	−0.41	—	—	−0.47
		D	−0.19	—	—	−0.22
		E	−0.71	—	—	0.07
		Mean ± 2 SE	−0.19 ± 0.32	—	—	−0.20 ± 0.32
IB	1979	A	−0.01	0.37	0.45	—
		B	−0.63	−0.08	−0.12	—
		D	0.10	−1.19	−0.92	—
		E	0.46	−0.81	−0.14	—
	1980	A	−0.77	−0.11	−0.62	—
		B	−0.82	−0.34	−0.34	—
		D	0.41	−0.18	−0.27	—
		E	−0.12	−0.26	0.64	—
		Mean ± 2 SE	−0.17 ± 0.44	−0.33 ± 0.44	−0.17 ± 0.44	—
	1981	A	−0.07	—	—	−0.67
		B	0.62	—	—	−0.03
		C	−0.08	—	—	0.06
		D	−0.03	—	—	0.11
		E	0.07	—	—	−0.37
		Mean ± 2 SE	0.10 ± 0.25	—	—	−0.18 ± 0.25
II	1979	A	−0.46	−0.05	0.04	—
		B	−0.57	−0.48	−1.14	—
		D	0	−0.98	−0.80	—
		E	−0.53	−0.31	−0.69	—
	1980	A	−1.42	−1.00	−0.55	—
		B	−1.28	−.061	−1.16	—
		D	0.31	−1.04	−0.75	—
		E	−0.85	−0.10	−0.47	—
		Mean ± 2 SE	−0.60 ± 0.46	−0.57 ± 0.46	−0.69 ± 0.46	—
	1981	A	−1.04	—	—	−1.48
		B	−0.76	—	—	−1.25
		C	−0.69	—	—	−0.48
		D	−0.76	—	—	−0.58
		E	−0.77	—	—	−0.36
		Mean ± 2 SE	−0.81 ± 0.26	—	—	−0.83 ± 0.26

Although the numbers Vincent (1987) presented lead to a general impression that hatchery trout had a negative effect on age 2 and older brown and rainbow trout, we have trouble accepting his conclusions without reservations because (1) the study had design deficiencies, (2) stream flow may have been a factor, and (3) large fish are not usually displaced by smaller fish. The stocking treatments were replicated in time, but the study lacked control sections for an adequate time period to provide evidence that the changes in abundance in the treatment sections were not part of an overall trend or normal annual fluctuations (e.g. Allen 1951; Burnet 1959; Libosv'arsky' 1973; Hunt 1974; Mense 1975; Avery and Hunt 1981). For the limited

TABLE 11. Mean instantaneous growth rates (in weight) for yearling and overyearling wild rainbow trout were recaptured from their initial section in September–October (number of nonmigrants weighed in June and fall in parentheses) in stocked and unstocked sections, Big Springs Creek, 1979–81.

Year	Stream zone	Section: Not stocked	50 trout stocked	100 trout stocked	400 trout stocked
		Yearling			
1979	A	0.95 (17)	0.77 (16)	0.58 (31)	—
	B	0.54 (13)	0.35 (14)	0.61 (20)	—
	D	−0.70 (24)		−0.26 (16)	—
	E	0.17 (1)		0.10 (5)	—
1980	A	1.26 (4)	0.53 (6)	0.69 (10)	—
	B	0.16 (5)	0.65 (49)	0.72 (13)	—
	D	1.21 (8)	0.06 (4)	1.46 (3)	—
	E	0.93 (1)	0.96 (2)	2.98 (4)	—
	Mean ± 2 SE	0.32 ± 0.28	0.49 ± 0.33	0.58 ± 0.24	—
1981	A	0.91 (11)	—	—	0.80 (5)
	B	1.46 (5)	—	—	1.09 (2)
	C	1.40 (5)	—	—	1.46 (4)
	D	0.99 (8)	—	—	1.37 (4)
	E	1.63 (4)	—	—	0.24 (7)
	Mean ± 2 SE	1.17 ± 0.31	—	—	0.87 ± 0.38
		Overyearling			
1979	A	0.65 (25)	−0.08 (12)	0.06 (19)	—
	B	−0.12 (13)	−0.19 (13)	0.04 (19)	—
	D	−0.37 (30)	0.07 (1)	−0.56 (11)	—
	E	−0.53 (2)	0.17 (11)	0.42 (7)	—
1980	A	0.44 (6)	−0.12 (4)	0.15 (9)	—
	B	−0.09 (9)	0.19 (9)	0.02 (24)	—
	D	0.77 (14)	0.92 (4)	0.76 (12)	—
	E	1.62 (3)	0.63 (4)	1.46 (8)	—
	Mean ± 2 SE	0.20 ± 0.20	0.11 ± 0.26	0.20 ± 0.19	—
1981	A	0.09 (8)	—	—	0.23 (7)
	B	0.75 (11)	—	—	0.74 (5)
	C	0.85 (14)	—	—	0.68 (6)
	D	1.15 (6)	—	—	0.83 (10)
	E	0.52 (10)	—	—	0.24 (4)
	Mean ± 2 SE	0.67 ± 0.14	—	—	0.58 ± 0.17

periods when data were collected in control sections, changes in trout abundance appeared similar to us in both treatment and control sections. Vincent (1987) dismissed the effect of flow in the Madison River on trout numbers, and we suspect he was right in doing so, but there were troublesome significant correlations between flow and fish numbers (the increased spring abundance of trout following years of no stocking in the Madison River coincided with the highest winter flows). Finally, we have trouble visualizing wild trout forced from their established positions by hatchery trout that were intruders and generally smaller than the age 2 and older wild fish. In the Montana study, the density of hatchery fish was less than $1.6 \cdot 100\ m^{-2}$ at time of stocking in the Madison River and $13 \cdot 100\ m^{-2}$ in O'Dell Creek. By fall, hatchery trout densities had declined to less than $1 \cdot 100\ m^{-2}$ and few survived to the following spring. The stocking rates were about half the wild trout density (age 1+) in the Madison River and 1.5 times the density of wild brown trout in O'Dell Creek. We have difficulty believing that the relatively low density of hatchery trout present in the Montana streams could cause the purported large declines in wild trout, even though the distributions of fish in the streams were probably clumped.

In addition to our studies in the St. Joe River and Big Springs Creek, Pollard and Bjornn (1973) also observed that rainbow trout stocked in an infertile stream containing juvenile steelhead rarely interacted with or displaced the wild trout. Miller (1954, 1958), in studies of the effects of stocked cutthroat trout on wild cutthroat trout in Gorge Creek, Alberta, found that wild trout were able to outcompete the stocked trout without incurring increased mortality.

If the abundance or production of trout in streams is regulated by density-dependent factors (LeCren 1965; Allen 1969; McFadden 1969), stocked trout would not have an effect on wild trout unless they both used the same space and food resources and those resources were in short supply. In the St. Joe River, we observed that most of the stocked trout did not occupy the same portions of pool and run habitat as the wild trout (Fig. 7). Other investigators have observed hatchery trout

TABLE 12. Mean condition factors for yearling and overyearling wild rainbow trout that were recaptured from their initial section in September–October (number of nonmigrants measured in parentheses) in stocked and unstocked sections, Big Springs Creek, 1979–81.

Year	Stream zone	Section: Not stocked	50 trout stocked	100 trout stocked	400 trout stocked
		Yearling			
1979	A	1.08 (17)	1.02 (16)	1.00 (31)	—
	B	1.04 (13)	1.04 (14)	1.00 (20)	—
	D	0.90 (24)	0.92 (22)	0.97 (16)	—
	E	1.05 (1)	0.90 (7)	0.99 (5)	—
1980	A	0.99 (4)	0.91 (6)	1.01 (10)	—
	B	1.05 (5)	1.00 (4)	0.97 (13)	—
	D	1.04 (8)	0.90 (4)	1.23 (3)	—
	E	1.16 (1)	0.98 (2)	1.17 (4)	—
	Mean ± 2 SE	1.00 ± 0.05	0.97 ± 0.05	1.01 ± 0.04	—
1981	A	1.08 (11)	—	—	1.07 (5)
	B	1.02 (5)	—	—	1.06 (2)
	C	1.03 (5)	—	—	1.16 (49)
	D	1.08 (8)	—	—	0.99 (4)
	E	1.03 (4)	—	—	0.83 (7)
	Mean ± 2 SE	1.06 ± 0.05	—	—	0.99 ± 0.06
		Overyearling			
1979	A	1.09 (25)	0.98 (12)	0.98 (19)	—
	B	1.05 (13)	1.05 (13)	1.08 (19)	—
	D	0.97 (30)	1.04 (8)	0.99 (11)	—
	E	0.97 (2)	1.00 (11)	1.10 (7)	—
1980	A	0.98 (6)	0.88 (4)	0.92 (9)	—
	B	1.03 (9)	0.99 (6)	1.01 (24)	—
	D	1.05 (14)	1.00 (4)	1.11 (12)	—
	E	1.14 (3)	1.10 ((4)	1.16 (8)	—
	Mean ± 2 SE	1.03 ± 0.01	1.01 ± 0.01	1.04 ± 0.01	—
1981	A	0.96 (8)	—	—	1.06 (7)
	B	1.04 (11)	—	—	1.14 (5)
	C	1.04 (14)	—	—	1.08 (6)
	D	1.07 (6)	—	—	1.05 (10)
	E	1.01 (10)	—	—	0.93 (4)
	Mean ± 2 SE	1.02 ± 0.03	—	—	1.05 ± 0.04

occupying sites that appeared to be less favorable than those used by wild trout (Vincent 1960; Pollard and Bjornn 1973; Soziak et al. 1979; Dickson and MacCrimmon 1982; Bachman 1984), a factor that may contribute to the high observed mortality of stocked trout. In Big Springs Creek, stocked trout were usually found in pools, sometimes near large wild trout. Subyearling and many of the yearling wild trout were found in the margins of pools and runs and in riffles filled with aquatic macrophytes, sites not used by the hatchery trout.

In addition to the incomplete overlap of habitat use by hatchery and wild trout, the question of space or food being in short supply must be addressed. In the St. Joe River, where cutthroat trout abundance was relatively high in 1979 after recovery from excessive exploitation (Fig. 5), doubling or quadrupling densities by stocking trout did not reduce abundance of wild trout. In Big Springs Creek, we did not see evidence of hatchery trout affecting wild trout until we stocked 400 trout per section, a 4- to 10-fold increase in density of larger trout. In the Madison River (Vincent 1987), densities of age 2 and older wild brown and rainbow trout were much higher in 1975 and 1976 when there was no stocking (combined average 1526 lb·mi^{-1}) than in 1967–69 (514 lb·mi^{-1} including the stocked trout). We could argue that the river was at only one-third carrying capacity in 1967–69, for whatever reason, and that adding hatchery trout at the rate of 800 lb·mi^{-1} should not have caused a shortage of space and food resources.

Our findings of hatchery trout having little effect on wild trout are consistent with the expectation that hatchery trout would not be able to displace wild trout of equal or larger size through agonistic behavior. If hatchery trout were to have a detrimental effect on wild trout, we might expect the smaller fish to be affected, but such was not the case in either ours or Vincent's (1987) studies. If Vincent's (1987) conclusions are true that hatchery trout decreased the abundance of age 2 and older wild trout (mostly larger than the stocked trout), the mechanism must be something other than one-on-one interactions. Butler (1975) drew a speculative parallel to Selye's (1973) theory on General Adaptation Syndrome in humans to suggest

that wild trout were stressed by hatchery trout, but to our knowledge no one has assessed socially induced stress in wild trout or the effects of such stress, if present. Eijke and Schreck (1980) reported that juvenile coho salmon (*Oncorhynchus kisutch*) with low hierarchical status in laboratory aquaria had higher stress levels (plasma cortisol). Noakes and Leatherland (1977) found a similar low position – high stress condition for hatchery rainbow trout, but dominant fish also had signs of stress. Bachman (1984) interpreted signs of dark, blotchy color and exhaustion as evidence of stress in wild brown trout after prolonged (3.5 min) agonistic encounter with hatchery brown trout, but he did not indicate that the interactions lead to mortality or displacement. Conceivably, large wild trout that would have no trouble dominating an individual hatchery trout might leave in frustration after trying to defend a territory or dominant role in a large school of "socially unaware" hatchery trout. We did not see evidence of such behavior by cutthroat trout in the St. Joe River.

In summary, we do not propose that managers can stock hatchery trout in streams without concern for the wild fish, but we do not believe that stocked trout, by themselves, will decimate adapted naturally reproducing populations of trout. In general, we believe that streams capable of supporting wild trout, particularly native trout, should be managed to enhance and use the natural production.

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Functional Maturity in Small Male Snow Crabs (*Chionoecetes opilio*)

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The mean size of male snow crabs (*Chionoecetes opilio*) in sexual pairs during the annual spring breeding migration to shallow water in Bonne Bay, Newfoundland, decreased from 118.6 mm carapace width (CW) in 1983 to 100.3 mm in 1987. This decrease is due to an increase in males <95 mm CW participating from 1.5% in 1983 to 32.3% in 1987. This change appears to have resulted from a reduced abundance of commercial size (≥95 mm CW) males due to a rapid development of an illegal fishery on this previously unfished population and, as a consequence, less competition between males for possession of females. The percentages of spermathecae containing new spermatophores for females paired with males <95 mm CW (67%) and those with males ≥95 mm CW (79%) were not significantly different. We assumed that each female with new spermatophores had recently mated with the male with which it was paired. Observations on selected pairs in captivity showed that males <95 mm CW are capable of mating with both primiparous and multiparous females. Our results indicate that small mature males can replace large males in breeding activity in a snow crab population. We conclude that in the male-only snow crab fishery in Atlantic Canada with a minimum legal size of 95 mm CW, population reproductive potential is maintained at a high level despite high exploitation rates on males ≥ 95 mm.

La taille moyenne de crabes des neiges mâles (*Chionoecetes opilio*) qui s'accouplent pendant la migration annuelle du printemps vers les eaux peu profondes de la baie Bonne à Terre-Neuve est passée de 118,6 mm (largeur de la carapace) en 1983 à 100,3 mm en 1987; cette diminution est attribuable à une augmentation du nombre de mâles géniteurs de moins de 95 mm de carapace, la proportion allant de 1,5 % en 1983 à 32,3 % en 1987. Ce changement semble provenir de l'abondance réduite de mâles d'une grosseur commerciale (carapace d'une largeur de 95 mm ou plus) en raison du développement rapide d'une pêche illégale de cette population autrefois inexploitée et, en conséquence, d'une moins forte compétition entre les mâles pour les femelles. Le pourcentage de spermathèques contenant de nouveaux spermatophores chez les femelles accouplées à des mâles dont la carapace avait moins de 95 mm de largeur (67 %) et celui des femelles accouplées à des mâles ayant une carapace de 95 mm ou plus (79 %) n'était pas significativement différent. Nous avons supposé que chaque femelle ayant de nouveaux spermatophores avait récemment copulé avec le mâle avec lequel elle s'était accouplée. Des observations de certains couples en captivité ont montré que des mâles de moins de 95 mm sont capables de s'accoupler avec des femelles primipares et multipares. Nos résultats révèlent que des mâles matures de petite taille peuvent remplacer des mâles de grande taille lors de la reproduction au sein d'une population de crabes des neiges. Nous en venons à la conclusion qu'une population de crabes des neiges dont les mâles de plus de 95 mm sont fortement exploités maintient son potentiel reproducteur dans les pêcheries de crabes des neiges mâles uniquement de la région atlantique du Canada où la largeur légale minimale de la carapace est de 95 mm ou plus.

Received March 9, 1988
Accepted August 11, 1988
(J9631)

Reçu le 9 mars 1988
Accepté le 11 août 1988

In the management of the Atlantic Canada snow crab (*Chionoecetes opilio*) fishery, it has been assumed that despite high levels of exploitation, reproductive potential in a stock remains at prefishery levels. The basis for this assumption is that females are protected from exploitation by the 95-mm carapace width (CW) minimum legal size because they never reach that size and males reach sexual maturity over the 51–72 CW range (Watson 1970). Elner and Robichaud (1983) reviewed the efficacy of the minimum legal size regulation and concluded that there was no evidence the assumption was invalid. However, Conan and Comeau (1986) stated that there is no evidence that sublegal males can mate in nature with multiparous females and concluded that the assumption was invalid.

Taylor et al. (1985) and Hooper (1986), based on observations and sampling in 1982, 1983, and 1984, described various behavioral, ecological, and biological aspects of an annual spring breeding migration to shallow water by sexually paired snow crabs in Bonne Bay, Newfoundland. There has been no

commercial snow crab fishery in Bonne Bay and the population was considered to be in a virgin state in 1984 (Taylor et al. 1985). All paired females examined in 1984 were multiparous (hard-shelled females that have produced more than one egg clutch). Each female had recently hatched a clutch of eggs or was carrying or in the process of hatching well-developed eggs (Taylor et al. 1985) which had been extruded about 1 yr previous (Paul 1984). Each female also had a ripe ovary indicating extrusion of a new clutch of eggs to be imminent.

Hooper's (1986) observations indicated that pair formation, in which the male grasps the female firmly by the walking legs on one side with a cheliped, occurs prior to movement into shallow water and that males carry and retain possession of individual females for extended periods (possibly up to 2 mo). While in shallow water there is keen competition between single and paired males for possession of females (Hooper 1986). Under prefishery conditions this competition may eliminate small, mature males from participating in breeding activity. In 1983, only one male in 67 pairs and in 1984 only three males in 304 pairs collected during the breeding migration were smaller than 95 mm CW; the smallest was 89 mm. It was unknown whether males smaller than these were capable of successful mating (i.e. functional maturity) in the absence of competition from larger males.

Since 1984, snow crabs in Bonne Bay have been exploited and the abundance of large crabs appears to have been greatly reduced. In research fishing, catch rates of commercial males dropped from 32.3 per trap haul in May–June 1985 to 1.9 during the same period in 1987 and the incidence of males <95 mm in diver-caught pairs collected during the breeding migration was considerably higher in recent years than previously observed (Ennis et al. 1988). This paper presents data collected during the breeding migrations in 1985, 1986, and 1987, along with some collected previously, as well as observations on pairs held in cages and aquaria. Our purpose is to consider the question of functional maturity in small male snow crabs and whether sublegal males are capable of maintaining reproductive potential at a high level in a heavily exploited population.

Materials and Methods

Sexually paired snow crabs were collected by SCUBA diving at depths from 10 to 30 m during the annual (April–May) breeding migration to shallow water in Bonne Bay each year from 1984 to 1987. In most cases, each pair was kept in a separate mesh bag. Maximum CW of each crab was measured to the nearest 1.0 mm and shell condition was determined according to criteria described by Miller and O'Keefe (1981). In females, the presence of old and new grasping marks was noted. These marks are made on the legs of females during the mating embrace. Those left from a previous mating are darkened and readily distinguishable from fresh marks of a recent mating (Paul 1984). The egg masses of females were examined to approximate the extent of hatching; for those in which hatching had been completed, the presence of newly extruded (bright orange) eggs was noted. Some females were also frozen and dissected in the laboratory to determine the presence of old and new spermatophores in the spermathecae. Distinguishing features of old and new spermatophores were described by Adams and Paul (1983), Taylor et al. (1985), and Paul (1984).

Male and female crabs were obtained by trapping at depths >60 m in Bonne Bay in early May 1986 around the time that the breeding migration to shallow water was starting. Selected male/female pairs were kept separately in cages on the bottom at 15 m and in onshore tanks supplied with running seawater. The females were examined in detail for evidence of successful mating after 2–3 wk. Selected pairs were also maintained separately in seawater facilities at the Northwest Atlantic Fisheries Centre in St. John's during 1987. Crabs for the latter observations were trapped in Conception Bay in November 1986 and males were held separately from females until paired.

Results

Size of Sexually Paired Males

The mean size of males in sexual pairs collected during the breeding migration in Bonne Bay has decreased significantly ($P < 0.001$) in recent years from 118.6 mm CW in 1983 to 100.3 mm in 1987 (Table 1). A Tukey test (Zar 1984) revealed that mean sizes were similar from 1983 through 1985 but that mean size in 1986 was significantly smaller than in 1983 and mean size in 1987 was significantly smaller than in all previous years. The percentage of males <95 mm CW in these pairs increased significantly ($P < 0.001$) from 1–2% in 1983–85 to 6.7% in 1986 and 32.3% in 1987 (Table 1). A Tukey-type test revealed that the 1986 percentage was significantly higher than that for 1984 but neither was different from those for 1983 and 1985. The percentage for 1987 was significantly higher than for each of the previous years. The paired males <95 mm CW collected in 1987 ranged from 62 to 93 mm CW ($\bar{x} = 79.5$, $N = 10$). The smallest paired male previously observed was 76 mm in 1985 (Table 1).

In the 1987 sample, the mean size of females paired with males <95 mm CW was 68.3 mm ($N = 10$) which was not significantly different from the 70.7 mm ($N = 21$) mean size of females paired with males ≥95 mm ($P = 0.24$). In the 1984–87 samples combined, there was also no significant difference ($P = 0.16$) in mean sizes (68.1 and 70.0 mm) of females paired with these male size groups.

In only two of the 616 pairs collected from 1983 to 1987 were the males smaller than the females with which they were

TABLE 1. Carapace widths of sexually paired male and female *C. opilio* collected by SCUBA divers in Bonne Bay, Newfoundland, 1983–87.

		Males			Females	
Year	*N*	$\bar{x}$ ± SD (mm)	Range	% <95 mm	$\bar{x}$ ± SD (mm)	Range
1983	67	118.6 ± 7.6	94–136	1.5	70.6 ± 5.3	55–85
1984	304	116.6 ± 8.3	89–140	1.0	70.0 ± 5.6	55–86
1985	199	113.8 ± 9.5	76–132	2.0	69.9 ± 5.0	60–90
1986	15	111.1 ± 9.9	94–123	6.7	69.1 ± 4.4	64–82
1987	31	100.3 ± 17.4	62–126	32.3	69.9 ± 5.2	58–82

TABLE 2. Percentage of spermathecae containing new spermatophores in sexually paired female *C. opilio* collected by SCUBA divers in Bonne Bay, May–June, 1984–87.

	Females with males <95 mm CW		Females with males ≥95 mm CW	
Year	*N*	% new sperm	*N*	% new sperm
1984	—	—	42	93
1985	2	50	45	80
1986	1	0	10	80
1987	9	78	21	76

TABLE 3. Observations on external egg masses (%) of sexually paired female *C. opilio* collected by SCUBA divers in Bonne Bay, May–June, 1984–87.

Male CW	*N*	None hatched	Some hatched	All hatched	All hatched and extruding new eggs
<95 mm	17	18	12	64	6
≥95 mm	492	49	14	36	1

paired. Both were taken in 1987. In one pair the CWs were 62 and 66 mm and in the other were 67 and 73 mm for the male and female, respectively. In the latter female, the spermatheca contained old and new spermatophores indicating recent mating. In all other pairs the male was at least 5 mm CW larger than the female.

Reproductive Condition of Sexually Paired Females

All sexually paired females collected during the breeding migration were multiparous carrying well-developed or hatching eggs. Among females whose spermathecae were examined, 67% ($N = 12$) of those paired with males <95 mm contained new spermatophores compared with 79% ($N = 76$) for those paired with males ≥95 mm in the same (1985–87) samples (Table 2). The difference was not significant ($\chi^2_c = 0.328$, $P > 0.50$). Observations on external egg masses of females also indicated no negative effects on hatching of old eggs or extrusion of new eggs (which occurs before pair separates) associated with being paired with small males. Among 17 females paired with males <95 mm in the 1984–87 samples, hatching and extrusion had progressed to an extent comparable with that for females paired with males ≥95 mm (Table 3).

Observations on Cage- and Tank-held Pairs

All of the male/female pairs which were selected from trapped samples and held in cages and in tanks at Bonne Bay (Table 4) involved females carrying old eggs. Examination of 17 similar females from the same samples revealed that none had new spermatophores in their spermathecae. When examined after being held for 2–3 wk, percentages of females with new spermatophores for those held with males <95 mm CW were not significantly different from those held with males ≥95 mm for either the cage-held ($\chi^2_c = 0.143$, $P > 0.50$) or tank-held ($\chi^2_c = 0.028$, $P > 0.75$) pairs (Table 4). Among the tank-held animals, grasping was observed in 75% of the pairs involving males <95 mm and in 83% of those involving males ≥95 mm; actual copulation was observed in 25 and 17%, respectively (Table 4).

Ten male/female pairs in which the males ranged from 62 to 75 mm CW were held in tanks at the Northwest Atlantic Fisheries Centre. These had been selected from trapped samples in which males and females had been held separately for 1–12 mo before pairing. Three of these involved females that had molted immediately before pairing. Copulation was observed in two of these, all three extruded new eggs, and of the two females whose spermathecae were examined, both contained new spermatophores only. The remaining seven pairs involved females that were carrying or in the process of hatching old eggs. One of these pairings took place in 3 mo, the others in 11–12 mo, after the females had been obtained from the wild. The spermathecae of all seven females contained old and new spermatophores when examined after being paired for a few days up to 3 wk.

Discussion

Our results indicate that male snow crabs smaller than the 95 mm CW minimum legal size applied in the fishery in Atlantic Canada are capable of mating with multiparous females in the wild. In Bonne Bay, the incidence of males <95 mm CW participating in the annual breeding migration to shallow water increased in 1986 and 1987. This appears to have been a direct result of reduced abundance of commercial-size males caused by the rapid development of an illegal fishery and, as a consequence, less competition between males for possession of females.

In addition to their participation in the annual breeding migration which occurs in Bonne Bay, there is other evidence that male *C. opilio* <95 mm CW are capable of successfully mating with multiparous females. Among the diver-caught, sexually paired females collected in Bonne Bay from 1985 to 1987, there was no difference in the proportion of spermathecae containing new spermatophores (indicating mating since pair formation) among the females examined (all of which were multiparous) that were paired with males <95 mm and those paired with males ≥95 mm. Similarly, in male/female pairs selected from trapped samples obtained at depths >60 m during the early part of the breeding migration to shallow water and held in cages and in tanks at Bonne Bay, there was no difference between females paired with males <95 mm and those with males ≥95 mm in the proportion of spermathecae with new

TABLE 4. Observations on male/female pairs of *C. opilio* selected from trapped samples and held in cages and in tanks at Bonne Bay in 1986.

	Pairs with males <95 mm CW				Pairs with males ≥95 mm CW			
	N	Grasping observed	Copulation observed	% with new spermatophores	*N*	Grasping observed	Copulation observed	% with new spermatophores
Cage-held	10[a]	—	—	30	8	—	—	50
Tank-held	12[b]	9	3	50	6	5	1	67

[a]$\overline{CW}$ males 88.1 mm, range 80–93 mm.
[b]$\overline{CW}$ males 82.6 mm, range 73–92 mm.

spermatophores when examined after 2–3 wk in captivity. Successful mating also occurred between selected pairs involving males <95 mm and both primiparous and multiparous females that were held in tanks at the Northwest Atlantic Fisheries Centre. Among tank-held pairs, precopulatory grasping was commonly observed and copulation was observed for several different pairs including some in which the males were <95 mm.

In the laboratory, Watson (1972) observed male *C. opilio* as small as 60 mm CW mating with primiparous females. In the closely related species *C. bairdi*, Adams and Paul (1983) also mated primiparous females with males ranging in size from 65 to 140 mm CW. They reported that all females received enough sperm to fertilize a full clutch of eggs, and following extrusion, 93% of the females had enough sperm remaining in their spermathecae to fertilize additional egg clutches.

In this study, we did not determine whether eggs extruded by females that had recently mated with males <95 mm CW were fertilized. Females in the genus *Chionoecetes* can fertilize one or more clutches of eggs using stored sperm which remains viable for up to 2 yr after mating (Watson 1972; Paul 1984). In *C. bairdi*, viable egg clutches were produced by 100% of the females that mated just prior to egg extrusion and by 97 and 71% of those utilizing stored sperm to fertilize one or two successive egg clutches, respectively (Paul 1984). In our samples, it would not have been possible to determine whether newly extruded eggs had been fertilized by sperm from the recent or a previous mating.

Taylor et al. (1985) found 1.4% of the paired females collected in Bonne Bay during the 1984 breeding migration to be carrying dead eggs. Among 54 eyed-egged females trapped at depths >110 m in Bonne Bay during late April 1988, none carried more than a few dead eggs scattered through the clutch (unpubl. data). This indicates that the incidence of nonfertilization of entire egg clutches in the wild is low.

Conan and Comeau (1986) concluded that there is no evidence that sublegal (i.e. <95 mm CW) males can mate in nature with multiparous females. We cannot be certain that the new spermatophores found in the spermathecae of diver-caught females paired with males <95 mm CW in Bonne Bay resulted from mating with those particular males. However, our observations on selected pairs held in cages and tanks show that males <95 mm are capable of mating successfully with multiparous females and we believe that this occurs in the wild as well.

We conclude that sublegal-size males can substitute for larger commercial-size males in normal mating activity in a *C. opilio* population. Our results indicate that reproductive potential (i.e. annual production of fertilized eggs) in a snow crab population will be maintained at a high level despite removal by fishing of a large proportion of commercial-size males each year. Although snow crab fishery management in Atlantic Canada has assumed this to be the case, there is no reason to suspect that recruitment to the fishery would be adversely affected if reproductive potential were not maintained near its maximum. It is reasonable to suggest that recruitment might be increased if reproductive potential were reduced. Morgan et al. (1982) found a much higher abundance of *Panulirus cygnus* puerulus (settling stage) being produced in years when abundance of spawning females was low. Also, there are major fluctuations in recruitment in the male-only *Cancer magister* fishery and Botsford's (1986) analyses indicate that there would be less variability if females were also harvested.

From the available evidence, we conclude that production of viable eggs, even in heavily fished *C. opilio* stocks, is maintained to the right of the ascending limb of a stock–recruitment curve whatever its general shape might be for this species.

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Shell Etching on Clams from Low-Alkalinity Ontario Lakes: A Physical or Chemical Process?

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Hinch, S. G., and R. H. Green. 1988. Shell etching on clams from low-alkalinity Ontario lakes: a physical or chemical process? Can. J. Fish. Aquat. Sci. 45: 2110–2113.

Elliptio complanata (Unionidae: Bivalvia) were sampled from sites of low and high water turbulence in four Ontario lakes that varied in pH (6.0–7.5) and alkalinity (22–243 $\mu eq \cdot L^{-1}$). External shell etching on each clam was quantified using an image analysis digitizer system. Within-lake variation in shell etching was contrasted using a log–log analysis of covariance approach. Clams exposed to higher turbulence regimes (and large sediment particles) were significantly more etched than low-exposure clams ($P < 0.05$). Approximately 52% of the total shell etching variability resulted from within-lake differences between high- and low-exposure clams. The remaining variation was accounted for by differences among individual clams. Shell etching in these lakes appears to be primarily a physical process probably related to water turbulence. Etching was not related to variation in lake water chemistry and thus is likely not influenced by lake acidification.

On a prélevé des échantillons d'*Elliptio complanata* (bivalves, unionidés) dans des emplacements de faible et de haute turbulence dans quatre lacs ontariens dont les eaux étaient d'un pH et d'une alcalinité de valeurs différentes (pH de 6,0 à 7,5, alcalinité de 22 à 243 $\mu eq \cdot L^{-1}$). On a ensuite fait l'étude quantitative du relief externe de chaque coquillage au moyen d'un système d'analyse d'image par numérisation. On a étudié la variation du relief des coquilles des bivalves d'un même lac par analyse de covariance bilogarithmique. On a constaté que les bivalves exposés à des eaux turbulentes (et à l'action de grosses particules de sédiments) avaient un relief significativement plus marqué que ceux qui étaient moins exposés ($P < 0,05$). Approximativement 52 % de la variabilité totale du relief résulte de différences entre les bivalves très exposés et peu exposés d'un même lac. Le reste de la variation s'explique par des différences individuelles. Il semble que le relief des coquilles des bivalves des lacs étudiés soit principalement le résultat d'un processus physique qui est probablement lié à la turbulence des eaux. Selon les observations, le relief n'est pas lié à la variation de la chimie de l'eau et, dès lors, il n'est probablement pas influencé par l'acidification du lac.

Received June 30, 1987
Accepted August 10, 1988
(J9336)

Reçu le 30 juin 1987
Accepté le 10 août 1988

Freshwater unionid clams possess shells that are primarily composed of calcium carbonate (Wilbur 1964) and that can be important in a freshwater system's calcium budget. Green (1980) found that 50% of the calcium in a shield lake near Inuvik, Northwest Territories, was in shells of live *Anodonta grandis*. The majority of this calcium is not returned to the lake until death of the clam and the subsequent dissolution of the shell. However, unionids also lose calcium while they are alive.

The unionid shell is covered by a thin fibrous schleroproteinaceous layer called the periostracum, which provides the matrix for deposition of calcium carbonate (Saleuddin and Petit 1983). The resilience of the periostracum seems to decrease on older portions of the shell, as it is often missing from the umbonal region. Underlying shell layers, primarily calcium carbonate, are also often missing.

It has been suggested that external shell etching (the loss of periostracum and underlying shell layers) is a chemical process analogous to chemical weathering of calcareous rock by naturally occurring carbonic acids (Beauchamp 1886; Grier 1920; Coker et al. 1921). However, the phenomenon of shell etching has never been quantitatively evaluated. Hence, an alternative explanation for shell erosion in living clams is physical erosion, through contact with abrasive particles. Just as in geological weathering, an interaction may exist between physical and chemical processes.

An added component to the question of the physical versus chemical basis of shell etching is the effect of anthropogenic enhancement of the acidity of precipitation. If the chemical weathering scenario is correct, then increases in acid loading (and decreases in lake pH) should enhance rates of shell etching just as it enhances strictly geochemical weathering. Alternatively, if shell etching is a predominantly physical process, etching will be independent of lake pH. In this study we examined shell etching in freshwater unionid mussels (*Elliptio complanata*) collected from four lakes which vary in alkalinity and pH. Within each lake, clams were collected from an area of high exposure to potentially abrading particles (high water turbulence) and low exposure (low water turbulence) based on sediment characteristics. Within-lake variation in shell etching due to exposure differences was compared across the four lakes.

Methods

The four study lakes are located in south-central Ontario on the Precambrian Shield (Fig. 1). Although morphologically

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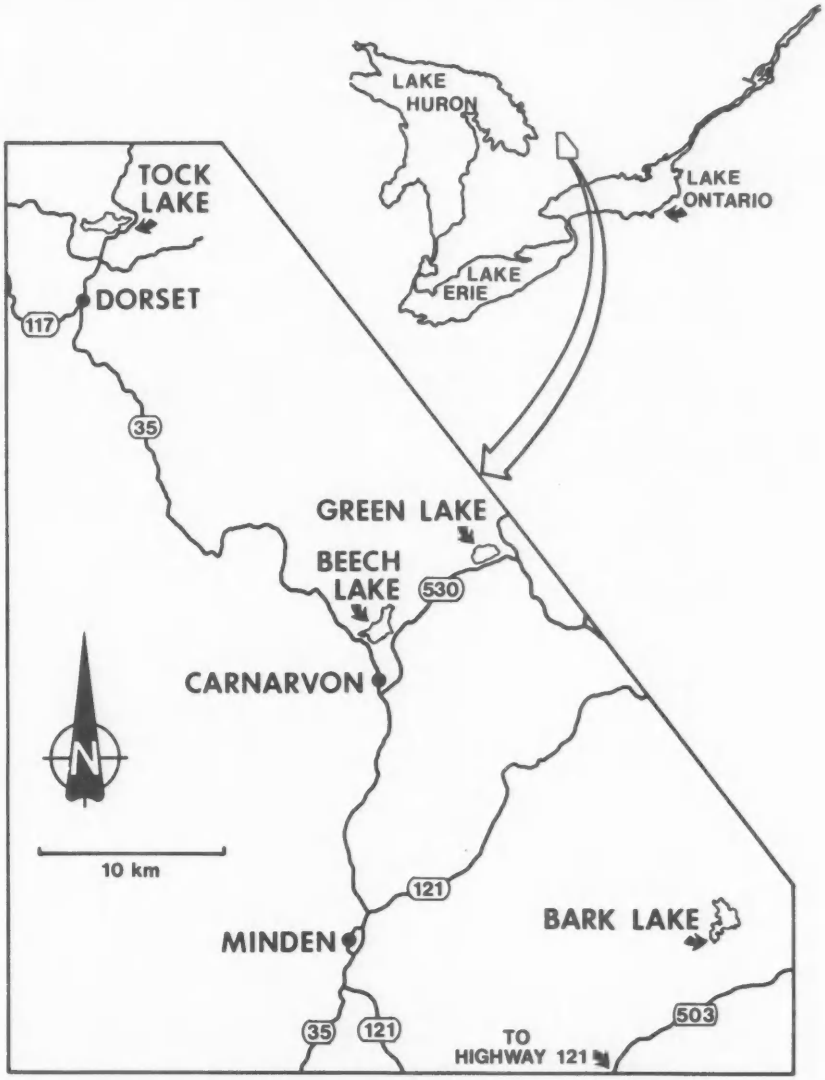

FIG. 1. Location of study areas in Haliburton Co., south-central Ontario.

similar (Hinch and Bailey 1988), the lakes were chosen because of differences in alkalinity and pH (see Fig. 2). Mean wind direction for the ice-free season is from the west-southwest, based on Environment Canada wind summary data from 1983 and 1984. Therefore, high-exposure habitats will exist primarily on the northeast shores of these lakes whereas low-exposure habitats will primarily be found on the southwest shores. In August 1985, 25 clams were collected using SCUBA from a low (southwest) and a high (northeast) exposure site in each lake (Fig. 2). Stretches of shoreline near inflows and outflows were not sampled. Sediments at the low-exposure sites appeared to be relatively fine whereas those at the high-exposure sites were primarily composed of sand and small gravel. These differences were quantitatively verified by randomly taking three sediment core samples at each site following the methodology of Allison and Harvey (1981) (Table 1). Clams occurred at water depths of 1.5–2.5 m. To reduce the effects of age structure as a confounding factor, only old individuals (greater than 8 yr of age based on annual rings) were collected. These clams tend to be the largest in the populations and are very near their population asymptotic sizes (S. G. Hinch, unpubl. data).

The meat was removed from each clam and the shells allowed to dry. Shell etching was quantified using an image analysis process run on a microcomputer-based digitizer system. Detailed descriptions of the design, construction, specifications, and software of the system can be obtained through correspondence with Dr. Lewis Brown (Solar Energy Research Institute, 1617 Cole Blvd., Golden, CO 80401, USA, unpubl. data). Shell etching was defined as any area on the shell which was missing periostracum and underlying shell layers (Fig. 3). In preparation for the digitizing process, all etched areas were painted white. This procedure provided a sharp contrast with

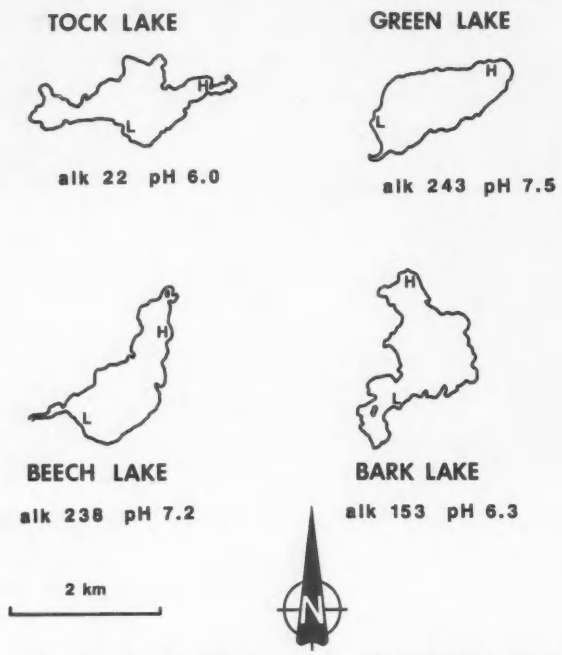

FIG. 2. Low-exposure (L) and high-exposure (H) sites in the four study lakes. Alkalinity ($\mu eq \cdot L^{-1}$) and pH values based on acidic precipitation in Ontario study (OME 1981–83).

TABLE 1. Mean (SE) percentage of sediment, by weight, that passed through a 0.5-mm sieve from each of the two exposure sites within the four lakes. At each site, $n = 3$.

Site	Mean (SE)
Green high	68.70 (5.25)
Green low	97.16 (1.01)
Beech high	66.27 (4.14)
Beech low	99.75 (0.16)
Bark high	55.00 (6.26)
Bark low	82.20 (2.66)
Tock high	50.58 (11.14)
Tock low	97.90 (0.31)

the dark brown nonetched areas. Nonetched shell areas were first determined. Both valves of a given shell were placed under the video camera with the outer shell surface up. The dark area was digitized and its area determined. The digitizing process only determines area (i.e. two dimensions). However, *E. complanata* have a thin profile (valve thickness ranging from 7 to 9 mm. Hinch and Bailey 1988), and we believe distortion caused by shell width was minimal. Total shell area was then determined by painting the shell's entire external surface black and repeating the digitizing process. Etched area was equal to the total shell area minus the nonetched shell area.

To account for population-dependent differences in clam sizes for a given age (Hinch and Bailey 1988), some form of standardization was required. To avoid the statistical problems inherent with ratios (Green 1979), we decided to use a regression of log of etched area (LEA) against the log of nonetched area (LNEA) rather than the superficially more simple percentage of shell etched. The advantage of a regression-derived estimate is that proportions can be compared without resorting

FIG. 3. *Elliptio complanata* shells from the (a and c) low-exposure and (b and d) high-exposure sites in Beech Lake demonstrating (a and b) maximum and (c and d) minimum amounts of shell etching.

TABLE 2. Probability levels for main and interaction effects from the ANCOVAs contrasting low- and high-exposure sites within the four lakes. For an experiment-wise error rate of $\alpha = 0.05$, the appropriate α for each test is 0.013 based on Bonferroni's inequality. Asterisks denote values that are not associated with null hypotheses of interest.

	Beech Lake	Green Lake	Bark Lake	Tock Lake
LNEA	*	*	*	*
SIDE	<0.001	<0.001	0.009	<0.001
LNEA × SIDE	0.024	0.267	0.008	0.022

to ratio-based variables (Green 1987). We then used analysis of covariance (ANCOVA) to compare the regression relationships from the high- and low-exposure sites within a given lake. Nested random effects ANCOVA was used to estimate the proportion of the total variance in shell etching attributable to variation among the four lakes, between exposure level sites within the lakes, and among individual clams within the sites. Maximum experimental error was controlled by adjusting the significance levels for independent, simultaneous comparisons using Bonferroni's inequality (Snedecor and Cochran 1980).

Results and Discussion

The effect of exposure (SIDE) was significant in the four analyses, indicating that exposure influences the percentage of shell that is etched (Table 2). The high-exposure clams were proportionately more etched than the low-exposure clams in each lake (Table 3). Comparisons of the interaction term (LNEA × SIDE) from the four analyses (using Bonferroni's inequality) showed that only Bark Lake was even marginally significant (Table 2). This result indicates that as clam size increases, the amount of etching does not proportionately

TABLE 3. Mean (SE) shell etched area (mm^2) from each of the two exposure sites within the four lakes. At each site, $n = 25$.

Site	Mean (SE)
Green high	638.03 (77.47)
Green low	277.74 (34.95)
Beech high	1216.83 (98.43)
Beech low	429.08 (76.65)
Bark high	475.84 (45.55)
Bark low	368.86 (26.05)
Tock high	801.77 (57.95)
Tock low	300.33 (29.33)

change between the two sides in Beech, Green, or Tock Lake. Only in Bark Lake is there evidence that exposure may influence the relationship between clam size and percentage of shell that is etched.

Components of variance computed from the nested ANCOVA indicate that 52.3% of the total shell etching variability resulted from differences between exposures (within-lake). Lakes did not differ significantly in degree of shell etching. The remaining 47.7% was accounted for by differences among individual clams within sites.

Shell etching does not appear to be related to differences in lake water chemistry (i.e. alkalinity and pH as others have proposed (Grier 1920; Coker et al. 1921; Tevesz and Carter 1980). Among-lake differences in shell etching variability were trivial. If water chemistry were important in influencing shell etching, one would expect etching to be "ordered" along a water chemistry gradient, with clams from Tock Lake the most etched and those from Beech and Green lakes the least. This ordering did not occur; in fact, clams from Beech Lake were the most etched. The majority of the variability in etching was explained by within-lake exposure differences. Therefore, we suggest that shell etching is mostly a physical process related to water turbulence.

The mechanism of physical shell etching is probably a "sandblasting" effect, as abrasive sediment particles move past shells or dislodged shells are scoured as they contact the substrate. The magnitude of shell etching should be greater on clams from exposed habitats because more sediment particles would be suspended in wave-washed, turbulent habitats. Also, shell dislodgement and subsequent scouring would also be expected to occur more frequently in more turbulent habitats. Etching is most commonly observed in the umbo (hinge) region of the shell. This pattern is not surprising because the umbo is the oldest and presumably weakest portion of the periostracum. It is also the shell portion most exposed to water movement, since younger portions of the shell are normally buried in the sediment.

Within each lake, percent shell etching remains the same as clams size increases for both high- and low-exposure clams despite the fact that high-exposure clams from each lake have a much faster growth rate than the corresponding low-exposure clams (Hinch and Bailey 1988). The exception to this was Bark Lake clams. Upon further investigation it was determined that as high-exposure Bark Lake clams grew in size, the absolute area of shell that was etched decreased. Although unionids can superficially reseal holes in their shells and can reinforce etched areas using adventitious conchiolin (Tevesz and Carter 1980), it is unlikely that they can replace nacreous shell layers and periostracum on their umbo. We remain unable to explain this etching pattern. Nonetheless, the overwhelming factor influencing shell etching in Bark Lake clams is exposure.

We have determined that shell etching on clams from four Ontario lakes, differing in pH and alkalinity, is a physical process related to substrate type and water turbulence. Laboratory studies using unetched clams, standard size sediment particles, and controlled water turbulence are needed to better assess the manner in which shell layers are physically removed.

Acknowledgements

The Ontario Ministry of the Environment and Environment Canada provided important data for this research. Lewis Brown's digitizing system was an invaluable component of this research. Robert Bailey, Keith Somers, and two anonymous reviewers have provided useful comments on earlier versions of this manuscript. This study was funded by NSERC and Ontario Ministry of the Environment operating grants to R.H.G. and an Ontario Graduate Scholarship to S.G.H.

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Mitochondrial DNA Diversity among Broodstocks of the Lake Trout, *Salvelinus namaycush*

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Grewe, P. M., and P. D. N. Hebert. 1988. Mitochondrial DNA diversity among broodstocks of the lake trout, *Salvelinus namaycush*. Can. J. Fish. Aquat. Sci. 45: 2114–2122.

Mitochondrial DNA was purified from 151 lake trout (*Salvelinus namaycush*) representing nine brood stocks along with an additional 30 fish from two natural populations. Eighteen restriction endonucleases were employed to analyze 126 brood stock fish. Two of these enzymes (Hind III and Bam HI) were used to examine the fish from the natural populations (Hare Island (Lake Superior) and North Knife Lake (Manitoba)) and the remaining brood stock samples from the Manitou (12 fish) and Seneca (13 fish) strains. The mitochondrial genome of the lake trout was 16 800 ± 200 base pairs in length. A single heteroplasmic individual was discovered in the Manitou strain. It contained two genomes, the less frequent of which was 20 base pairs shorter than the other. The 18 restriction enzymes resolved 13 mitochondrial clones which fell into three major groups. These clonal groups, which can be identified by their Bam HI restriction phenotypes, have a specific distribution: a western Great Lakes group, a central Great Lakes group, and an eastern Great Lakes group. Seven mitochondrial clones were unique to a particular stock. In addition there were dramatic shifts in the relative proportions of the six remaining mitochondrial clones among the brood stocks. These results indicate that mitochondrial DNA markers have great potential for the identification and management of lake trout strains.

L'ADN mitochondrial a été purifié dans 151 touladis (*Salvelinus namaycush*) représentant neuf stocks géniteurs ainsi que chez 30 autres poissons provenant de deux populations naturelles. Dix-huit endonucléases de restriction ont été utilisées pour analyser 126 poissons du stock géniteur. Deux de ces enzymes (Hind III et Bam HI) ont servi à examiner le poisson provenant des populations naturelles (du Hare Island (lac Supérieur) et du North Knife Lake (Manitoba)) et les autres échantillons des stocks géniteurs des souches de Manitou (12 poissons) et de Seneca (13 poissons). Le génome mitochondrial du touladi était au total de 16 800 plus ou moins 200 paires de base. Un seul individu hétéroplasmique a été découvert dans la souche de Manitou. Il contenait deux génomes, dont le moins fréquent comptait 20 paires de base de moins que l'autre. Les 18 enzymes de restriction ont produit 13 clones mitochondriaux qui appartenaient à trois groupes principaux. Ces groupes de clones qui peuvent être identifiés par les phénotypes de restriction obtenus au moyen de la Bam HI ont une répartition spécifique : un groupe de l'ouest des Grands Lacs, un groupe du centre des Grands Lacs et un groupe de l'est des Grands Lacs. Sept clones mitochondriaux étaient uniques à un stock particulier. De plus, la proportion relative occupée par les six autres clones mitochondriaux au sein des stocks géniteurs variaient de façon spectaculaire. Ces résultats indiquent que les marqueurs de l'ADN mitochondrial offrent de grandes possibilités pour l'identification et la gestion des souches de touladi.

Received March 11, 1988
Accepted August 11, 1988
(J9637)

Reçu le 11 mars 1988
Accepté le 11 août 1988

The lake trout (*Salvelinus namaycush*) fishery in the Great Lakes collapsed in the mid-1940's as a result of the sea lamprey (*Petromyzon marinus*), pollution, and overfishing (Eshenröder et al. 1984). Efforts to reestablish self-sustaining populations from hatchery stocks have intensified following control of the lamprey. In most cases, plantings include a variety of brood stocks that originated from different locations in the Great Lakes (Pycha and King 1985). Members of the various stocks can be differentially fin clipped to permit comparison of their survival success, but this does not allow assessment of their reproductive capability in the wild. Assessment of reproductive success requires the existence of genetic markers between brood stocks which will ideally persist over many generations.

During the 1970's, work on fish stock identification focused on electrophoretic studies because variation in allozyme patterns ordinarily has a simple genetic basis. Recent studies which have surveyed as many as 100 loci have been more successful (C. Krueger and B. May, Department of Natural Resources, Cornell University, Ithaca, NY, unpubl. data) in revealing genetic differences among lake trout stocks than earlier studies which were based on fewer loci. Yet even comprehensive allozyme surveys have failed to reveal evidence of significant genetic variation within some species. For instance, perch populations in Lake Michigan (Leary and Brook 1982) and ciscoes in Lake Superior (Todd 1981) were found to be nearly monomorphic at the loci examined. Even in the most successful cases, differences among populations cooccurring in a specific lake basin have been restricted to fairly minor shifts in gene frequency. Such variation provides an indication of population

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structure, but is not dramatic enough to permit assignment of individual fish to a specific stock on anything more than a probabilistic basis. Allozyme markers have proven more useful in certain other contexts, for example studying the fate of introduced fish or the incidence of introgression. Yet even in these cases, certain limitations exist. Allozymically marked fish can be distinguished from residents for only one generation and then only if fixed gene substitutions exist. Hybridization between the introduced and native stocks and segregation of variants make it difficult or impossible to determine the fate of the introduced fish in later generations.

It is now generally accepted that, when one is dealing with either conspecific or closely related species, restriction analysis of mitochondrial DNA provides more detailed information on phylogenetic relationships than do allozyme studies (Avise et al. 1979a, 1979b; Ferris et al. 1981; Lansman et al. 1983; Avise et al. 1984; Avise and Saunders 1984; Avise et al. 1986). There are two major reasons for this. First, mitochondrial studies permit examination of variation at the nucleotide level. By contrast, allozyme studies involve detection of variation in charge characteristics of gene products, and thus nucleotide substitutions which do not alter charge (about two-thirds) are overlooked. Second, the resolution capability of mitochondrial DNA studies is enhanced by the fact that mitochondrial DNA evolves at 10 times the rate of the nuclear genome (Brown et al. 1979).

In the context of fish stock identification, mitochondrial DNA has the additional advantage of being a maternally inherited asexual genome (Hutchinson et al. 1974; Francisco et al. 1979; Lansman et al. 1981). This has an important effect on the anticipated level of genetic divergence between stocks. The extent of genetic divergence in any segment of the genome is determined by the interaction between processes which promote divergence and the process of gene exchange which reduces divergence. When populations are large and selection pressures weak, the amount of gene exchange is critical in determining the extent of divergence. For nuclear genes this exchange can be accomplished by the movement of individuals of either sex. In the case where females are philopatric and males mate randomly, no divergence in the nuclear genome will occur. However, this same pattern of gene exchange permits the divergence of the mitochondrial genome because it is maternally inherited.

In summary, there are two main reasons to expect more clear-cut divergence among stocks of fish in the mitochondrial rather than the nuclear genome: the more rapid rate of evolution of mitochondrial DNA and the failure of male exchange to reduce genetic divergence among stocks.

It has recently become evident that studies of mitochondrial DNA diversity offer exceptional promise in the discrimination of fish stocks in a manner useful to fisheries management. Specifically, the studies carried out so far (Avise et al. 1984; Avise and Saunders 1984; Berg and Ferris 1984; Wilson et al. 1985; Avise et al. 1986; Grewe and Hebert 1987; Wilson et al. 1987; Billington and Hebert 1988) have demonstrated the occurrence of abundant mitochondrial DNA variation in a variety of fish species.

This study examines the value of mitochondrial DNA restriction fragment data in defining brood stocks of the lake trout. The study concentrated on nine brood stocks used in restocking programs in both Canada and the United States. The lake trout is a philopatric species (Swanson 1974) and thus has a population structure favoring the development of genetically isolated stocks. Yet, attempts to distinguish these stocks on morphological and allozymic criteria have met with limited success. Natural reproduction is now occurring in Lake Michigan (Jude et al. 1981), Lake Huron (Nester and Poe 1984), and Lake Ontario (Marsden et al. 1988) in areas where lake trout have been planted. It is essential to identify these natural recruits in order to determine which strains are reproducing successfully in the wild.

Materials and Methods

The lake trout strains analyzed in this study were obtained from hatchery brood stock, with the exception of the wild-caught Simcoe and Lewis Lake fish (Table 1). In addition to the hatchery strains, a limited survey of mitochondrial diversity was also carried out on lake trout from two natural populations, Hare Island (Lake Superior) and North Knife Lake (Manitoba) (Fig. 1).

Mitochondrial DNA (mtDNA) was extracted and purified according to the protocol outlined in Grewe and Hebert (1987). Liver tissue was routinely used, but immature ovaries and mature testes proved to be good sources of mtDNA as well. Fresh tissue was used exclusively, as yields of mtDNA from frozen tissue were substantially reduced. Liver samples were ordinarily processed within 4 d of dissection; however, samples could be kept on ice for 10 d without a reduction in mtDNA yield (pers. obs.).

Eighteen restriction endonucleases were employed to analyze all 126 brood stock fish. These included 14 six-base (Ava I, Bam HI, Bcl I, Bst EII, Eco RI, Hind III, Nco I, Pst I, Pvu II, Sal I, Sma I, Sst II, Xba I, Xho I), 2 five-base (Hinf I, Nci I), and 2 four-base (Hpa II, Taq I) endonucleases. Additional fish from the Manitou ($n = 12$) and Seneca ($n = 13$) strains, along with Hare Island ($n = 21$) and North Knife Lake ($n = 9$) fish, were analyzed using only Bam HI and Hind III. Samples were digested using the buffer system and incubation temperatures specified by the supplier (Bethesda Research Laboratories). Samples were then end-labelled with ^{32}P-radio-labelled nucleotides, using the fill-in reaction of the large (Klenow) fragment of DNA polymerase I (Maniatis 1982). Unincorporated nucleotides were then removed and the samples lyophilized. The samples were then reconstituted in 15 μL of buffer (8% sucrose and 0.05% bromophenol blue in TBE), split into 7.5-μL aliquots, and simultaneously electrophoresed utilizing agarose and polyacrylamide gels in a TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) buffer system. DNA fragments ranging in size from 20 000 to approximately 500 base pairs were resolved on 1.2% agarose gels, while fragments of between 1000 and 26 base pairs were resolved on 4% acrylamide gels (38:2 acrylamide – bisacrylamide).

Upon completion of electrophoresis, gels were dried onto a filter paper backing (3MM) and exposed overnight to X-ray film (Fuji RX). Restriction fragment sizes were estimated from the autoradiographs utilizing the program DNAGEL (Kieser 1984; modified by P. Grewe) run on an Apple II plus computer in conjunction with a HIPAD digitizing pad (model DT-11A, Houston Instruments).

Appropriate double digests were conducted to confirm restriction site homologies and to determine a restriction site map of the lake trout mitochondrial genome. The size of the lake trout mitochondrial genome was estimated by comparing the sums of restriction fragments produced by the 13 six-base enzyme digests.

TABLE 1. Lake trout populations surveyed.

Strain (abbreviation)	No.	Year class	Origin of fish and date obtained
Manitou (MAN)	4	1980	Maple Research Station, Ontario, May 1985
Big Bay (BIG)	5	1980	Maple Research Station, Ontario, May 1985
Simcoe (SIM)	10	—	Lake Simcoe, identified as Simcoe stock by personnel at Sibbald Point Assessment Unit, Feb. 1986
Killala (KIL)	11	1983	Hill's Lake Hatchery, Ontario, Aug. 1985
Seneca (SEN)	15	1978	Allegheny N.F.H., Nov. 1984
Green Lake (GLA)	9	1975	Jordan River N.F.H., Oct. 1984
	6	1975	Jordan River N.F.H., Oct. 1985
Lewis Lake (LEW)	8	1982	Jackson N.F.H., Aug. 1985
	9	—	Wild-caught fish from Lewis Lake, Sept. 1985
Clearwater Lake, Manitoba (CMA)	1	1982	Great Lakes Fish. Comm., June 1985
Marquette (SUP)	10	1975	Jordan River N.F.H., Nov. 1984
(domestic)	8	1981	Jordan River N.F.H., Nov. 1984
	20	1977	Jordan River N.F.H., Nov. 1985
Hare Island (HAR)	21	—	Hare Island reef, Lake Superior by M.N.R. personnel in Thunder Bay, Ontario, Aug. 1986
North Knife (NOR)	9	—	Collected from North Knife Lake, Manitoba, Aug. 1986

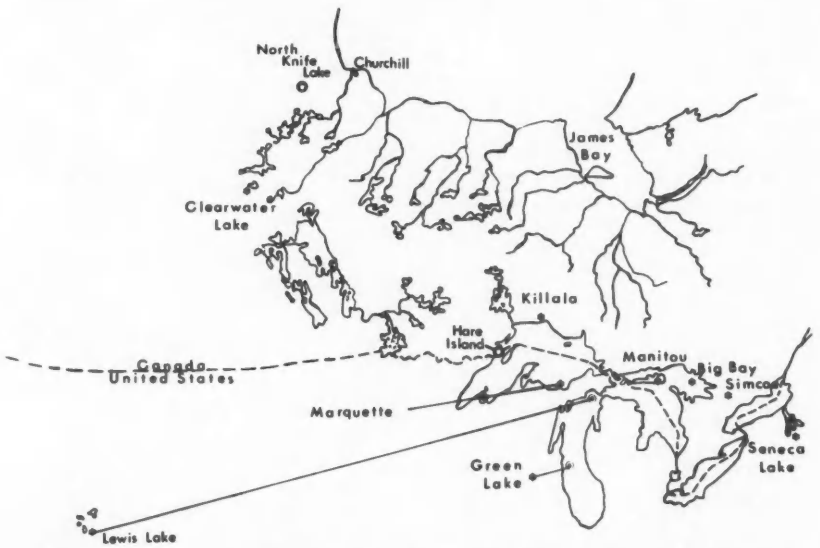

FIG. 1. Origin of the brood stocks and natural populations of lake trout used in this study.

Restriction patterns obtained for each of the 17 endonucleases were assigned a letter (A, B, C, etc.) in the order of their discovery. Each unique 17 letter combination described the "genotype" of a specific mitochondrial clone and these were subdivided into three groups on the basis of their Ava I and Bam HI restriction patterns. For example, a clone was labelled "A" if the Ava I or Bam HI A pattern was present. It was labelled "B" if the Bam HI B pattern was present and "C" if the Bam HI C pattern was present. Justification for the recognition of these three groups is provided later.

The effective number of clones in each stock was calculated using the inverse of Simpson's Index (Simpson 1949; Parker

TABLE 2. Restriction phenotypes of the thirteen mtDNA clones resolved by the 10 polymorphic restriction enzymes and their abundances among the nine brood stocks.

Clone	No. of observations	Ava I	Bam HI	Bst EII	Hind III	Nco I	Sma I	Hinf I	Nci I	Hpa II	Taq I
A1	40	A	A	A	A	A	A	A	A	A	A
A2	18	A	A	A	A	A	A	D	A	A	D
A3	5	A	A	A	A	A	A	A	C	C	A
A4	1	A	A	B	A	A	A	A	A	A	A
A5	1	A	D	A	A	A	A	D	A	A	D
A6	1	A	A	A	A	A	A	A	D	A	A
A7	1	A	A	A	A	A	A	D	A	A	A
A8	1	A	A	A	A	A	A	D	A	A	E
A9	1	A	A	A	B	A	A	D	A	A	D
B1	41	B	B	A	A	A	A	B	B	B	B
C1	13	C	C	A	A	A	A	C	B	B	C
C2	2	D	C	A	A	A	B	C	B	B	C
C3	1	C	C	A	A	B	A	C	B	B	C
Total	126										

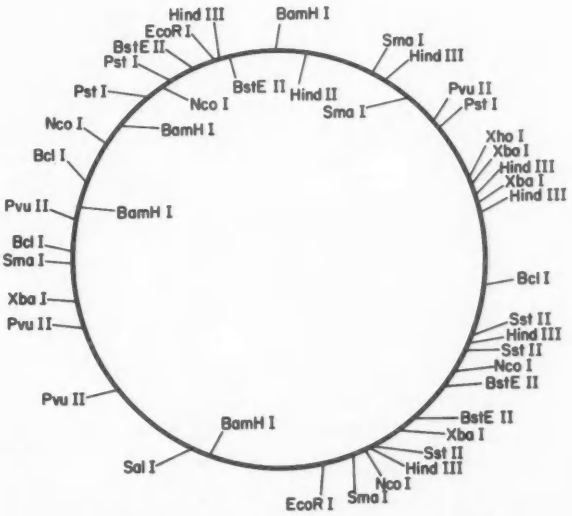

FIG. 2. Restriction site map of the lake trout mitochondrial genome with variable sites labeled inside the circle.

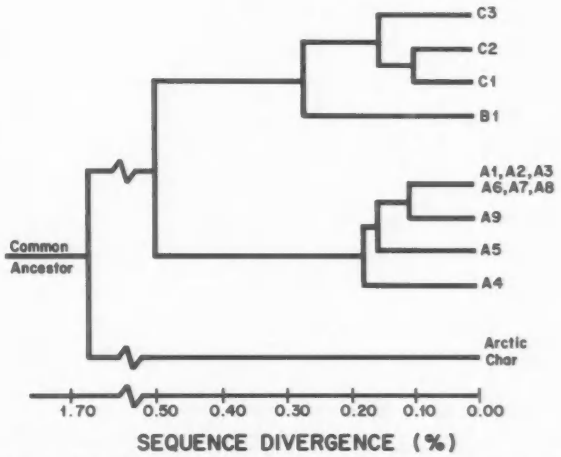

FIG. 3. UPGMA-generated phenogram based on the maximum likelihood estimate of the number of nucleotide differences per site (*d*), constructed from the six-base endonuclease data. Note: distance values have been plotted as 1/2*d*.

1979). The effective number of clones and actual number of clones detected in each brood stock were separately regressed against sample size using the General Linear Models Procedure of SAS.

The maximum likelihood estimates of the number of nucleotide differences per site (*d*) were calculated following Nei and Tajima (1983). Divergence estimates between taxa were reported as percent sequence divergence (*d*), while the distance phenogram, constructed by UPGMA (Sneath and Sokal 1973), was plotted with each lineage being exactly half this value (1/2*d*) between neighboring taxa (see Nei et al. 1985). Standard error values for the branching points of the distance phenogram were calculated by the method of Nei et al. (1985). Divergence times were estimated using the mitochondrial DNA divergence clock for primates (1% sequence divergence·million yr^{-1}·$lineage^{-1}$) calibrated by Brown et al. (1979). It was impossible to map the four- and five-base restriction sites. These results were therefore excluded from the sequence divergence analysis. Arctic char (*Salvelinus alpinus*) mtDNA, isolated from fish taken in the Tarsuk Arm (northwestern Baffin Island), was used to root the six-base UPGMA dendrogram.

Finally, a phenetic tree utilizing all restriction endonuclease patterns was constructed by parsimony. Assuming that parallel site gains and losses were extremely rare or did not occur, clones with the fewest restriction site changes between them were connected together. For example, to obtain the Bam HI C restriction type from the Bam HI A restriction type, the Bam HI B type was required as an intermediate.

Results

Lake Trout Mitochondrial Genome

The lake trout mitochondrial genome was estimated to be approximately 16 800 ± 200 base pairs in length. All mitochondrial genomes examined were identical in length with one exception. One fish from the Lake Manitou strain (clone

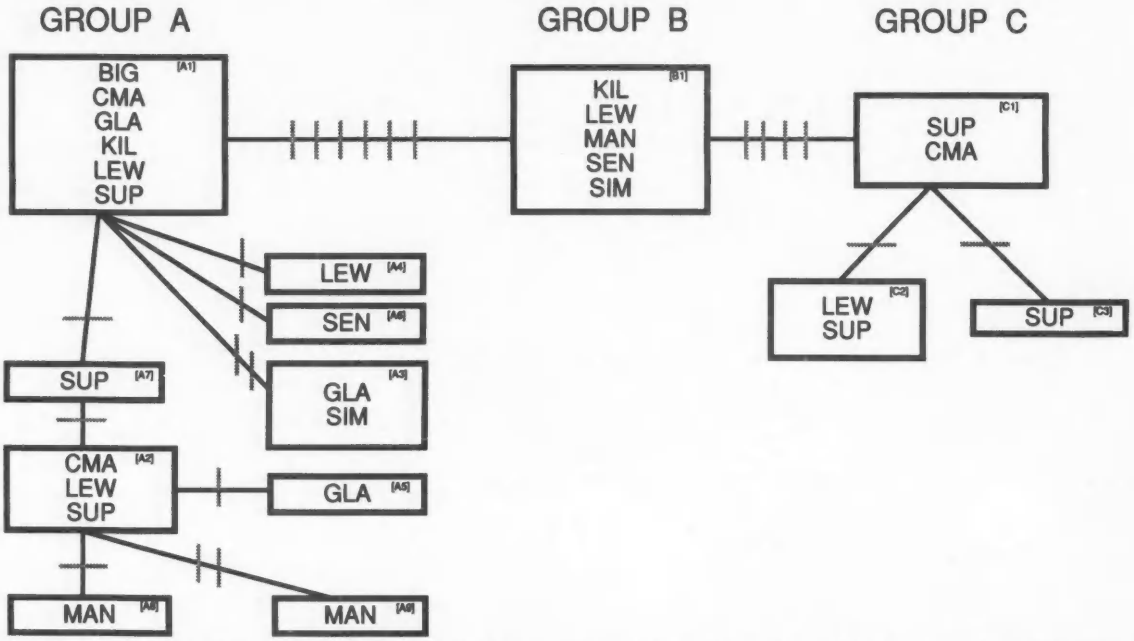

FIG. 4. Parsimony network of the 13 lake trout mitochondrial clones recognized by the 10 polymorphic restriction enzymes. Clonal designations (from Table 2) are indicated by the square brackets and dotted lines represent the number of restriction endonucleases differentiating each clone. The three-letter abbreviations (see Table 1) indicate the stocks containing each clone.

TABLE 3. Percent sequence divergence values (reported below the diagonal) and standard deviations (reported above the diagonal) among the lake trout and Arctic char mtDNA clones recognized by the six-base endonucleases.

	A1	A5	A4	A9	B1	C1	C3	C2	Char
A1	—	0.190	0.198	0.198	0.281	0.396	0.454	0.443	0.904
A5	0.197	—	0.281	0.281	0.344	0.443	0.497	0.485	0.934
A4	0.197	0.392	—	0.281	0.344	0.443	0.497	0.485	0.934
A9	0.197	0.392	0.392	—	0.344	0.443	0.497	0.485	0.934
B1	0.392	0.585	0.585	0.585	—	0.268	0.336	0.328	0.878
C1	0.775	0.964	0.964	0.964	0.375	—	0.185	0.181	0.936
C3	0.987	1.177	1.177	1.177	0.572	0.184	—	0.262	0.908
C2	0.964	1.150	1.150	1.150	0.559	0.180	0.366	—	0.963
Char	3.120	3.322	3.322	3.322	3.039	3.432	3.236	3.624	—

A9) was heteroplasmic, containing two mitochondrial genomes of different size (confirmed through double digests). Both long and short A9 genomes were characterized by a Hind III site gain with the smaller (by 20 base pairs) genome appearing less intense (approximately 1/10) on autoradiographs. The heteromorphic fragment, characterizing the smaller genome, was only detected with digests utilizing either Hind III or Hinf I. These digests presumably cut the two genomes into fragments which migrated to positions on the gels where they could be resolved. No other heteroplasmic individuals were observed among the remaining 125 fish surveyed for the entire 17 restriction enzymes.

Restriction Patterns

All fish possessed identical restriction patterns for eight of the endonucleases (Bcl I, Eco RI, Pst I, Pvu II, Sal I, Sst II, Xba I, and Xho I). The 10 remaining restriction endonucleases revealed polymorphic patterns and permitted the recognition of 13 mitochondrial clones (Table 2) among the 126 brood stock fish examined. Eight of these clones were resolved by six-base restriction endonucleases, but the others could only be recognized by utilizing four- and five-base restriction endonucleases. The restriction site map (Fig. 2) details the positions of both the variable and invariant sites (six-base enzymes data only).

Phylogeny of Mitochondrial DNA Types

Both sequence divergence and parsimony analysis were used to examine the phylogenetic relationships among the 13 mitochondrial clones. Genetic distance analysis, based on six-base enzymes (Fig. 3), showed that the 13 clones fell into three major groups (A, B, and C), with groups B and C being more closely related to each other than either was to group A. The average genetic distance between members of groups B and C was 0.0138, while the average distances between members of A and the B and C groups were 0.0238 and 0.0245, respectively.

The parsimony analysis (Fig. 4) shows the differences between the mitochondrial clones and the mutational steps

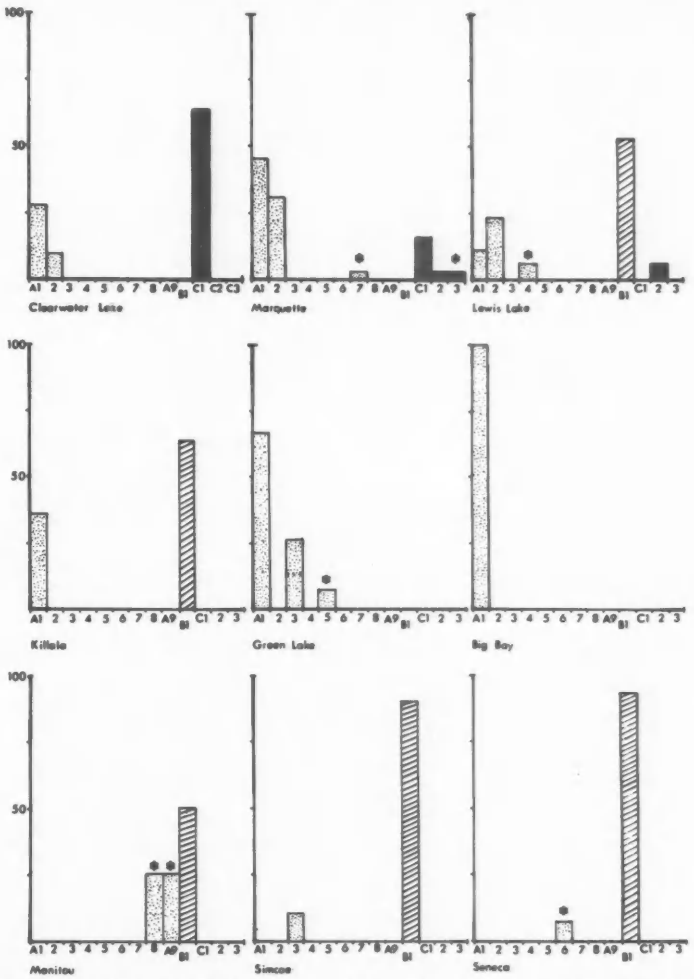

FIG. 5. Proportions of the 13 mtDNA clones found among the nine brood stocks surveyed (asterisks indicate clones unique to a particular strain).

required to move from one clone to the next. These results extend those gained from the distance analysis, as they include information on site changes at four- and five-base endonuclease sequences. The parsimony analysis clearly revealed that group B was intermediate to groups A and C. For example, to move from A to B required a gain of a Bam HI site, while moving from A to C required the gain of both this site and an additional Bam HI site. Group B was separated from members of group A by a minimum of six restriction site differences and from members of group C by at least four site differences. Thus, a minimum of 10 mutational steps were required to move from the A type to the C type.

Clonal Distribution

Clone group A was the most diverse of the three groups and included nine different clones, while groups B and C were represented by one and three clones, respectively. Clones A1, A2, B1, and C1 dominated (>13 fish per clone) the clonal assemblage, with approximately 90% of the fish belonging to one of these clones (Table 3). The remaining nine clones were rare, each being represented by fewer than five fish. In fact, seven of these nine clones were unique to a particular strain. They were found in the Green Lake (A5), Lewis Lake (A4), Manitou (A8 and A9), Marquette (A7 and C3), and Seneca Lake (A6) strains. There were dramatic shifts in the relative proportions of the six shared mitochondrial clones among the brood stocks (Fig. 5). The Clearwater Lake and Marquette strains contained the same three dominant clones (A1, A2, and C1), but the C1 clone was more common in Clearwater Lake. The Lewis Lake strain also contained the A1 and A2 clones, but the B1 and C2 clones were present, while the C1 clone was absent. The Green Lake strain also contained the A1 clone, but there was a high proportion of the otherwise rare A3 clone, and the B1 and C clones were absent. All fish from the Big Bay strain were found to possess the A1 clone, while the Killala Lake strain contained clones A1 and B1, with the latter making up the majority of this stock. Both the Simcoe and Seneca Lake strains contained high proportions (>90%) of the B1 clone, but the two stocks possessed different group A clones. The Manitou strain con-

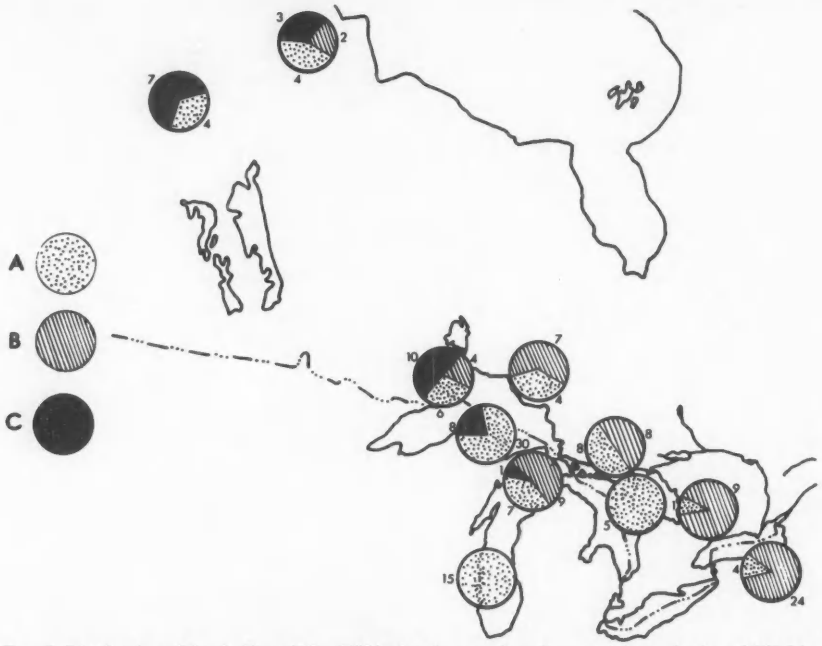

FIG. 6. Distribution of the A, B, and C mtDNA clonal groups based on a total sample size of 181 lake trout from the 11 populations surveyed.

tained a high proportion of the B1 clone (50%), together with two unique clones (A8 and A9).

Fish from the natural populations of Hare Island and North Knife Lake were only analyzed with Hind III and Bam HI to permit the assignment of these fish to one of the three major clonal groups. Both populations contained all three clonal groups, with A composing 33 and 44% in the Hare Island and the North Knife Lake samples, respectively. Group B clones composed 19 and 22% of the populations, while group C made up 48% of the Hare Island and 34% of the North Knife Lake samples.

The distribution of the A, B, and C clonal groups exhibited a geographical pattern (Fig. 6). Group C clones predominated in the northwestern section of the Great Lakes, while Group B clones predominated in the southeastern Great Lakes. Group A clones were found in every strain but were most common in the central Great Lakes region.

Clonal Diversity

Analysis of the effective number of clones per strain indicated that the Manitou, Lewis Lake, and Marquette strains showed the highest level of mitochondrial diversity, with approximately 3.0 clones per stock. The Green, Killala, and Clearwater Lake strains each contained 2.0 clones, while the Big Bay, Simcoe, and Seneca strains each contained approximately 1.0 clone. Analysis of variance of all populations sampled indicated that there was a significant direct relationship between the effective number of clones and total number of fish analyzed per stock ($p < 0.01$, $r^2 = 0.54$). An even stronger direct relationship was indicated between the actual number of clones detected and sample size ($p < 0.001$, $r^2 = 0.70$).

Discussion

The estimated length of the lake trout mitochondrial genome concurs well with estimates for other salmonid genomes (Berg and Ferris 1984; Birt et al. 1986; Thomas et al. 1986). This value is similar to those reported for other vertebrates which range from 15 to 20 kilobase pairs long (Gray 1982; Brown 1983). The lengths of the mitochondrial molecules were invariant in all lake trout examined with the exception of the single heteroplasmic fish from the Manitou stock. Although heteroplasmy is generally uncommon, it has been reported in holstein cows (Hauswirth et al. 1984), *Cnemidophorus* lizards (Densmore et al. 1985), *Rana* frogs (Monnerot et al. 1984; Bermingham et al. 1986), and in two fish species, the bowfin (*Amia calva*) (Bermingham et al. 1986) and the American shad (*Alosa sapidissima*) (Bentzen et al. 1988). Such length heterogeneity is thought to arise from insertions/deletions near the D-loop region which occur during replication of the mtDNA molecule (Brown 1983). Birky (1982) has suggested that heteroplasmy is ordinarily confined to germ cell lines, and becomes evident in somatic tissue only when more than one mtDNA molecule becomes established in the somatic cell line during differentiation of the embryo. Heteroplasmy was obviously not confined to the germ cell line in the Manitou fish, as liver tissue was used for extraction. Both size variants in this fish could be identified by an extra Hind III site which should allow easy recognition of its siblings in the Manitou population and further study of this case of somatic heteroplasmy.

The analysis of mtDNA diversity within strains of the alike trout, using Parker's (1979) "effective number of clones," showed that the Marquette, Lewis, and Manitou strains were the most diverse. However, as sample size varied among stocks and was shown to have a significant effect on the level of mitochondrial clonal diversity, these apparent differences among stocks must be interpreted with caution. Thus, the high diversity seen in the Marquette strain is likely, at least in part, a result of the large number of fish sampled ($n = 38$). However, it is noteworthy that the Marquette strain originated from fish

taken from three different locations in Lake Superior (Krueger et al. 1983). Similarly, fish stocked into Lewis Lake were reared from eggs obtained from several different reefs in Lake Michigan. The original population was also supplemented with fish of unknown origin. It is uncertain whether these later fish made a significant contribution to the Lewis Lake population (Vischer 1983), but certainly, opportunities existed for the inclusion of a large amount of mitochondrial diversity in this stock. The Manitou strain also seems to have a high amount of mitochondrial diversity, but, as only four fish were examined, more analyses are required before drawing further conclusions. The Green Lake strain contained three mitochondrial clones despite being confined to hatcheries for at least three generations (Krueger et al. 1983) and being subjected to several transfers with high mortality. It is interesting to note that the three brood stocks (Big Bay, Simcoe, and Seneca) with the lowest mitochondrial diversity (i.e. one effective mitochondrial clone) were each established from single small collections of females. For example, only 17 females founded the Seneca brood stock, while the Big Bay and Simcoe strains came from small original populations. The limited work on natural populations suggests that they have levels of mtDNA diversity similar to the most diverse of the hatchery strains examined.

Analysis of the extent of mitochondrial diversity is a complex issue, for the number of clones detected depends upon one's sampling intensity of the mitochondrial genome and upon the number of individuals examined. Clearly, two important questions need to be answered in order to estimate the amount of mtDNA variation in any lake trout population or brood stock. First, how many individuals must be analyzed to detect all the clones present in a specific stock? Second, what proportion of the mitochondrial genome needs to be surveyed in order to resolve all mitochondrial variants? The problem is analogous to that of estimating clonal diversity in asexual taxa, in which a combinatorial approach has been developed to study the relationship between the number of clones detected and both sample size and genome sampling intensity (Hebert et al. 1988). It is obvious that the present survey has not been extensive enough to detect all mitochondrial variants, but sufficient to make it clear that there are a small number of well-differentiated lake trout mitochondrial lineages whose frequencies vary among brood stocks and natural populations.

The analysis of genetic similarity among the 13 mitochondrial genotypes indicated that they could be divided into three groups, with clone groups B and C more closely related to each other than to A. Based on the rate of primate mtDNA evolution (Brown et al. 1979), the B and C lineages separated from the A lineage approximately 500 000 yr ago and separated from each other approximately 270 000 yr ago. By comparison, the Arctic char and lake trout lineage last shared a common ancestor about 1.67 million yr ago.

Distribution of the A, B, and C clonal types suggests that lake trout populations in the Great Lakes derive from three separate glacial refugia, which contained unique clonal groups. Before further conclusions can be drawn, additional evidence is required from natural populations, particularly from northern Canada along postglacial dispersion routes (Lindsey 1964; Bailey and Smith 1981; Black 1983). It is interesting to note that the geographical distributions of the A, B, and C mitochondrial clonal groups show some similarities, with patterns of gene frequency divergence at allozyme loci and chromosome banding patterns (Phillips and Ihssen 1987) observed among lake trout stocks.

In summary, we have shown that lake trout populations contain large amounts of mtDNA diversity. The level of mitochondrial diversity in some brood stocks appears lower than that in natural populations, but other brood stocks have maintained a normal level of diversity. In each brood stock there are a small number of common clones and a few individuals with closely similar genomes, which have apparently arisen as mutational derivatives. The frequencies of the common clone groups vary among brood stocks. In part, this variation is likely a consequence of founder effect during brood stock establishment or stochastic variation of mtDNA lineage extinction. However, congruence in the mitochondrial characteristics of brood stocks from specific sectors of the Great Lakes suggests that the variation among strains also reflects historic distributional patterns of mitochondrial variants. The mitochondrial variants are separable into three groups which likely differentiated from one another in separate glacial refugia and subsequently colonized the Great Lakes. The dominant clone group (A) was probably carried into the lakes by fish which spent the Pleistocene south of the ice sheets. The other two groups (B and C) apparently represent groups that were confined during the Pleistocene in eastern or western refuge(s).

Acknowledgments

We thank Tom Dowling and the personnel of Dr. Wes Brown's laboratory for their help with mtDNA techniques. We also thank Randy Eshenroder for his help in making contacts at hatcheries holding the fish stocks used in this survey. The following individuals provided fish for the study: Larry Wubbels and Howard Jackson (Marquette and Green Lake), David Ostergaard (Seneca), Peter Ihssen (Manitou and Big Bay), Dan O'Connor (Clearwater Lake), P. Graf (Killala Lake), and Matt Bernard (Lewis Lake), the personnel of the Sibbald Point Assessment Unit (collected and identified the Simcoe samples), Tammy Black (additional Seneca Lake brood stock samples), Ken Cullis and personnel of the Fisheries Assessment Unit in Thunder Bay (Hare Island population), and Brent Glynn (Arctic char from Baffin Island). We would also like to thank Dave Stanton and Neil Billington for their comments on earlier drafts of this manuscript and Jan Wilson for her technical support. This research was supported by grants from the Great Lakes Fishery Commission and the Natural Sciences and Engineering Research Council of Canada.

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Factors Affecting Survival and Cation Concentration in the Blackflies *Prosimulium fuscum/mixtum* and the Mayfly *Leptophlebia cupida* during Spring Snowmelt

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Hall, R. J., R. C. Bailey, and J. Findeis. 1988. Factors affecting survival and cation concentration in the blackflies *Prosimulium fuscum/mixtum* and the mayfly *Leptophlebia cupida* during spring snowmelt. Can. J. Fish. Aquat. Sci. 45: 2123–2132.

We used a transplant experiment to examine the effects of short-term pH depressions in spring on whole-body concentrations of nine metal cations (Ca, Mg, Na, Zn, Pb, Al, Mn, Cd, and Fe) and survival of two species of aquatic insects collected from two habitats with year-round differences in pH. Survival of *Leptophlebia cupida* and *Prosimulium fuscum/mixtum* was close to 100% in 4- and 10-d in situ toxicity tests prior to and during snowmelt at pH levels ranging from 6.5 to 4.2. Differences in metal concentrations (Zn, Cd, and Na) existed between mayflies collected from different streams with pH values of 6.2–5.6. Both species transplanted from pH 6.2 to 4.2 streamwater had significant decreases in whole-body concentrations of Ca, Al, and Mn. Additionally, mayflies had significant losses of Mg, Fe, and Pb, while blackflies decreased in whole-body Na and Zn concentration. Percentages of the whole-body Al concentration in the adsorbed, absorbed, and gut content fractions were 3.3, 31.7, and 65%, respectively, for *L. cupida* and 1.6, 56.4, and 42%, respectively, for *P. fuscum/mixtum*. The percent adsorbed doubled in transplanted (versus control) mayflies, but the overall whole-body Al concentration decreased.

Une expérience de transplantation nous a permis d'étudier les effets que la baisse passagère du pH au printemps peut avoir sur la concentration corporelle de neuf cations métalliques (Ca, Mg, Na, Zn, Pb, Al, Mn, Cd et Fe) et sur la survie de deux espèces d'insectes aquatiques dans deux types d'habitats dont le pH diffère toute l'année. On a constaté que la survie de *Leptophlebia cupida* et de *Prosimulium fuscum/mixtum* approche 100 % lors d'épreuves de toxicité *in situ* de 4 et 10 jours réalisées avant et durant la fonte des neiges alors que le pH du milieu se trouvait entre 6,5 et 4,2. On a constaté que chez des éphémères prélevées dans différents cours d'eau où le pH était de 6,2–5,6, la concentration de certains métaux (Zn, Cd et Na) était différente. Chez les insectes des deux espèces qu'on a capturés dans des eaux de pH de 6,2 pour les transplanter dans des eaux de pH 4,2, la concentration corporelle de Ca, Al et Mn a baissé de façon significative. En outre, la concentration de Mg, Fe et Pb a baissé dans une mesure significative chez les éphémères, tandis que chez les simulies, on a noté une baisse de la concentration corporelle de Na et Zn. On a mesuré la concentration corporelle d'Al dans les fractions adsorbées, absorbées et dans le contenu du tube digestif : les valeurs s'élèvent respectivement à 3,3, 31,7 et 65 %, dans le cas de *L. cupida*, et à 1,6, 56,4 et 42 %, dans le cas de *P. fuscum/mixtum*. La proportion adsorbée a doublé chez les éphémères transplantées (par comparaison au témoin), mais la concentration corporelle générale d'Al a baissé.

Received March 23, 1987
Accepted August 10, 1988
(J9186)

Reçu le 23 mars 1987
Accepté le 10 août 1988

Stream pH depressions during spring snowmelt have been reported in many locations in Scandanavia (Haapla et al. 1975; Gjessing et al. 1976; Hultberg 1976) and eastern North America (Jeffries et al. 1979; Johnson et al. 1981; Harvey and Lee 1982; Keller 1983). In surface waters flowing over the Canadian Shield, for example, pH levels in spring range from one to three units below the annual mean. This seasonal change in stream chemistry has been augmented in recent years by atmospheric deposition of metals on aquatic ecosystems far removed from emission sources (Dillon and Evans 1982; Evans and Dillon 1982; Evans et al. 1983; Johnson et al. 1986; LaZerte 1986). Since reduced pH during snowmelt increases mobili-

zation of both meteorologically and geologically derived metals (e.g. Al, Mn, Fe, and Zn; see Anonymous 1985), as well as base cations (e.g. Ca, Mg, and K) from soils and sediments (Likens et al. 1977; Hall et al. 1980, 1987; LaZerte and Dillon 1984), concentrations of these elements may be elevated 10–100 times that of background levels.

Loss of fish and other biota from soft waters (<2.0 mg Ca/L) has been attributed to elevated metal levels (Al, Fe, and Mn) during acidic snowmelt episodes (Schofield and Trojnar 1980; Harvey and Lee 1982; Andersson and Nyberg 1984). Fish mortality results in part from failure to regulate major whole-body ions (Na, K, and Ca) at toxic pH and metal levels (Wood and McDonald 1982; Cusimano et al. 1986). Aquatic insects have ionoregulatory mechanisms similar to those in fish (Komnick et al. 1972), but the links between transient and rapid changes in proton and metal concentrations in streamwater and the impact on survival and ionoregulation in stream insects are not well understood.

Whole-body concentrations in insects do not give a true picture of physiological and toxicological effects because of the varying amounts of metals that are either associated with sediments in the digestive tracts (Elwood et al. 1976; Smock 1983a, 1983b) or adsorbed to the surface of the organism relative to concentrations absorbed in body tissues (Kormonday 1965; Smock 1983b). Partitioning of these elements, specifically Al, is at least partially controlled by the size of the organism and kinds of particles ingested (Elwood 1976). However, little is known about the influences of short-term changes in water chemistry (particularly pH) on either whole-body cation concentrations or amounts of Al partitioned (adsorbed, absorbed, gut material) in invertebrates.

We hypothesized that (1) the large changes in water chemistry during snowmelt would cause reduced survival and alter ionoregulation in aquatic insects, (2) metals would accumulate in insects exposed to more acidic water, and (3) more Al would be adsorbed at lower pH. To test these hypotheses, we transplanted presumptively acid-sensitive insects (Hall et al. 1980) to study sites characterized by different pH regimes and intensely monitored water chemistry. We analyzed survival during exposure to the rapidly varying water chemistry as a measure of toxicity, whole-body cation concentration (particularly Na and Ca) as an index of change in ionoregulation and metal accumulation, and partitioning of Al as a possible factor affecting survival.

Study Areas

One inflow and two outflows of headwater lakes with different H^+ concentrations were selected for invertebrate studies: Plastic Inflow No. 1 (PCI, annual mean pH 4.5), Plastic Outflow (PCO, 5.6), and Harp Outflow (HPO, 6.2). These study lakes are on the Precambrian Shield in south-central Ontario

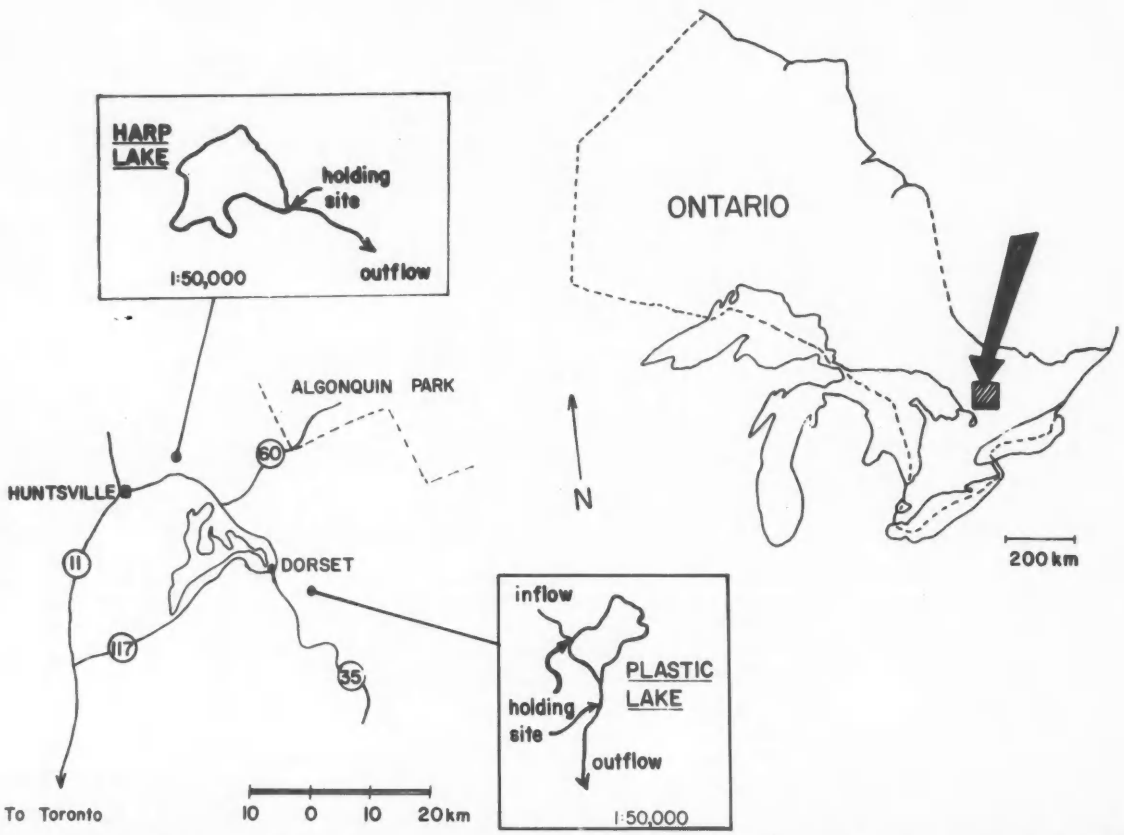

FIG. 1. Schematic representation of the three study locations (Harp Lake: latitude 45°23′, longitude 79°07′; Plastic Lake: 45°11′, 78°50′) in Haliburton County, south-central Ontario. Numbers in the circles represent highways.

TABLE 1. Summary of the design for the transplant experiments. The two transplants indicated by an asterisk were controls to test for mortality due to handling.

Source	Destination
Harp Outflow (HPO, pH 6.2)	Plastic Outflow (PCO, pH 5.6)
Harp Outflow (HPO)*	Plastic Inflow (PCI, pH 4.5)
Harp Outflow (HPO)	Harp Outflow (HPO)
Plastic Outflow (PCO, pH 5.6)	Harp Outflow (HPO, pH 6.2)
Plastic Outflow (PCO)*	Plastic Inflow (PCI, pH 4.5)
Plastic Outflow (PCO)	Plastic Outflow (PCO)

(Fig. 1). There are no point-source discharges into these lakes, except for seasonal use of cottages on Harp Lake. The major stream inflows and outflows of both lakes have been continuously monitored by V-notch weirs/flumes since 1979 (Scheider et al. 1983). Details of geology are provided in Jeffries and Synder (1983). Forest cover in the catchments consists predominantly of coniferous stands around Plastic Lake and deciduous stands around Harp Lake.

Materials and Methods

Organisms

We selected species of insects that are common in Muskoka–Haliburton streams. The mayfly *Leptophlebia cupida* (Say) is univoltine with up to 34 nymphal instars (Clifford et al. 1979). Eggs of this species hatch in late July, and the nymphs are found in pools where they feed on fine particulate detritus and grow rapidly until early November. Emergence of the subadults occurs from mid-May to early July.

The blackflies used in this study belong to the *Prosimulium hirtipes* (Fries) complex (*P. fuscum* Syme and Davies 1958 and *P. mixtum* Syme and Davies 1958). Individuals of *P. fuscum/mixtum* have a univoltine life cycle with seven larval instars (Davies and Syme 1958; Hayton 1979). Eggs are laid in May and June, and hatching occurs in autumn after a summer egg diapause. The time from hatching to pupation is approximately 150 d. Larvae filter and feed on fine particulate organic matter in stream riffles.

Transplant Experiments

Immature nymphs of *L. cupida* and larvae of *P. fuscum/mixtum* were collected from HPO and PCO but do not occur in PCI. Individuals of similar size were chosen for the transplant, whole-body ion concentration, and metal partitioning studies (mayfly total length, HPO: $\bar{x} \pm$ SE = 8.79 ± 0.059 mm, n = 314; PCO: 8.59 ± 0.01, 101; blackfly head capsule width, HPO: 0.71 ± 0.004, 385). The mayflies used were in the 26th (or greater) instar (Clifford et al. 1979), and blackflies were in the fifth to seventh instar (Hayton 1979). In the laboratory these organisms were maintained for no more than 24 h in water of the same temperature as encountered in the field. These two species were then transferred back to the field according to the design in Table 1.

The immatures were placed in screened cages (see Clark and Hall 1985 for details) with either 20 mayflies or 30 blackflies to a cage. Four or five cages for each species from HPO and for the mayfly from PCO were used in each transplant and were submerged individually in perforated plastic buckets, which protected the cages from disturbance from ice or fluctuating streamwater during snowmelt.

The organisms were checked daily. Survival was defined as active movement when the insects were probed with forceps. We conducted seven 4-d transplants and one 10-d reciprocal transplant between 25 February and the end of April (Fig. 2, arrows). Two exposures took place before and six during snowmelt. At the end of the exposure periods the organisms were frozen until cation analyses.

Water Chemistry

Water samples were collected every 3 h with an automated Isco sampler (model 1680). Sample bottles were soaked overnight in a mixture of 1 N NaOH and 0.1% sodium hypochlorite followed by rinses with double-distilled, deionized water. The bottles were then soaked a second night in 5 N HNO_3 and thoroughly rinsed before use. Calcium, Mg, Na, Fe, Al, Mn, and Zn were analyzed by flame atomic absorption (OMOE 1985). Alkalinity was determined by Gran titration. Dissolved organic carbon (DOC) was measured colourmetrically after the sample was oxidized in a UV digestor in an acid–persulphate medium. Hydrogen ion concentration was measured with a combination glass electrode standardized against appropriate buffers.

Whole-Body Ion Concentrations

Mayfly nymphs and blackfly larvae from the transplant experiments were thawed, fragmented with Teflon-coated forceps, placed in acid-washed (5% HNO_3) pre-weighed 10-mL Teflon vials, and weighed after drying at 60°C for 72 h. The insects were digested in 16N HNO_3 (Baker Instra analyzed) for 24 h at 100°C. A 0.25-mL aliquot of digestate was then added to 9.75 mL of ultrapure distilled water. Six acid and six double-distilled water blanks were prepared and run with each group of 20 samples, which were analyzed for Al, Na, Zn, Pb, Ca, Fe, Mg, and Mn with a flameless atomic absorption spectrophotometer (AAS). An oyster tissue standard (NBS) was used as a check for Cd and Zn only. The experimental values for Cd and Zn were within 0 and 16%, respectively, of the certified values (n = 7). The AAS detection limit for each of the metals was Zn = <5, Pb = 20, Cd = 5, Mn = 3, Fe = 20, Al = 10, Ca = 20, Mg = 5, and Na = 30 μg/L. Undetectable Pb concentrations (60% of the samples) were considered to be one half the minimum detectable concentration.

We used analysis of covariance (ANCOVA), with the $\log_e$-transformed biomass of the sample as the covariate and the actual amount of a given metal ($\log_e$-transformed) in the sample as the dependent variable. This procedure accounted for variation in metal concentrations among samples due to differences in weight of the organisms in the samples. For the blackflies, which were all from HPO, we included destination (HPO, PCO, or PCI) as a term in the ANCOVA model to see if where the insects were placed affected their final metal concentrations. For the mayflies, which came from either HPO or PCO, we included the source of the insects (PCO or HPO), destination (HPO, PCO, or PCI), and the interaction of "source" and "destination" in the ANCOVA model to see if where the insects came from affected their final metal concentrations and whether this effect depended on where the insects were placed.

SAS Procedure GLM (SAS Institute Inc. 1985) was used for all of the ANCOVA analyses. We generated LSMEANS (SAS Institute Inc. 1985) for each of the groups considered in the ANCOVA model and then divided these LSMEANS by the mean $\log_e$ biomass before plotting the back-transformed results using units of milligrams per gram. We used this procedure to

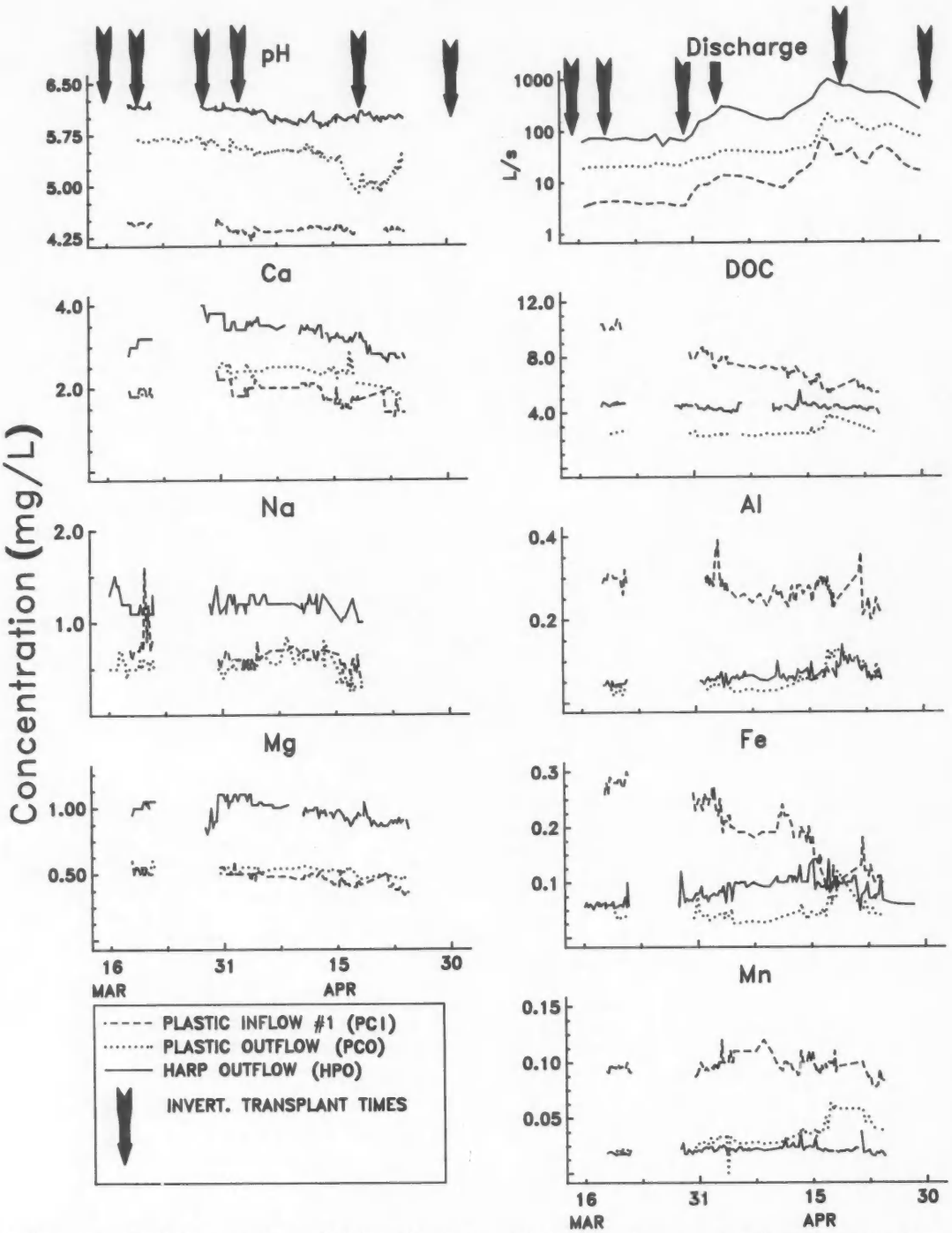

FIG. 2. Streamwater chemical concentrations and discharge for the three study locations. Arrows represent time of insect transplants. Two transplants (25 February to 1 March; 5–9 March) are not shown because no short-term (~3 h) chemical measurements were recorded.

express our results in concentration units because the ANCOVA essentially compared the amount of metal in a sample of the (geometric) mean sample biomass among the various groups.

Metal Partitioning

Aluminum partitioning (adsorption, absorption, gut contents) was evaluated on five groups of frozen HPO mayflies (20

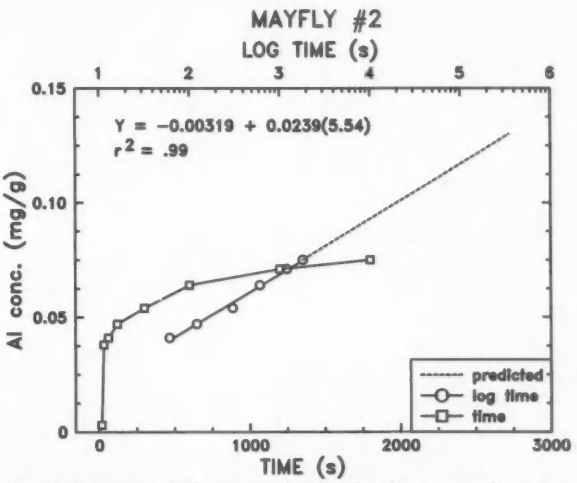

FIG. 3. Regression of Al concentration desorbed from *L. cupida* against time. For prediction of amount desorbed within 96 h, the time axis was log-transformed.

in each) and two groups of frozen HPO blackflies (30 in each) that had been transplanted to PCI, plus live organisms (controls) from PCO and HPO (30 blackflies per sample, 20 mayflies per sample) that had not been used in experimental transplants. To determine surface adsorption of Al, insects (frozen or live) were transferred with Teflon forceps into precleaned and preweighed vials (see whole-body analyses). Ten millilitres of 0.1 mM HNO_3 was added to each sample. Subsamples of 1.5 mL were removed at 15, 30, 60, 120, 300, 600, 1200, and 1800 s and placed into smaller vials for total Al analyses as outlined earlier. After removal of each 1.5-mL aliquot, the same amount of clean HNO_3 was immediately replaced to maintain 10 mL in the vial with organisms. This dilution was taken into account during calculation of metal concentrations. From our laboratory experiment we predicted the maximal amount of Al that would have desorbed in the 96-h transplant experiments at pH 4.1 by regressing $\log_{10}$ time versus Al desorbed (Fig. 3). To determine amount of Al in gut contents, 30 immature blackflies and 20 immature mayflies from both HPO and PCO were placed in petri dishes with approximately pH 6.2 HPO water for 96 h to allow them to clear their guts. These insects were then digested and analyzed following the procedures above for whole-body analyses.

Results

Water Chemistry

The basic geologic/physiographic differences between the stream systems lead to different pH and discharge regimes with important secondary effects on streamwater chemistry (Fig. 2). For Mn, Fe, Al, and DOC, the two outflow streams tend to be more similar than the inflow stream. Discharge, pH, Ca, Na, and Mg are more similar within the Plastic Lake catchment, with HPO having consistently higher values. The elevated acidity, metal cations, and DOC concentrations prior to snowmelt in PCI are characteristic of drainage water from a minerotrophic peatland (Gorham et al. 1984). The differences in Ca, Mg, and Na concentrations in HPO relative to PCO are believed to be due to the thicker overburden of the latter, since atmospheric deposition is similar for the two study areas.

The effects of variation in discharge were most evident in PCI. Iron and DOC declined in concentration as discharge increased, and Al peaked in concentration on the ascending limb of the stream hydrograph. These elements did not vary as much in the two outflow streams. Calcium and Mg in HPO likewise decreased in concentration as discharge increased.

Invertebrate Survival

All blackflies and mayflies survived (97–100%, data not shown) during seven of the 4-d exposures prior to and during snowmelt. However, during the one 10-d transplant, 20% of the blackflies collected from PCO and exposed to PCI died. Blackflies from HPO and mayflies from both outflows had 100% survival in PCI.

Whole-Body Ion Concentrations

In five of the nine cations considered (Al, Zn, Ca, Mn, and Na), the site to which the blackflies were transplanted (i.e. their "destination" PCO, mean pH 5.6; PCI, mean pH 4.5) had a significant ($p < 0.05$) effect on final concentrations. The size-corrected concentrations (i.e. body burden of the cation at the mean sample weight) showed a similar basic pattern for all of these cations (Fig. 4); those blackflies staying at HPO (all originally came from HPO) all had the highest concentrations, whereas the concentrations for those transplanted to PCO and PCI were either similar (Al, Ca, and Na) or higher for those moved to PCO (Zn and Mn). The pattern shown by those cations not significantly ($p > 0.05$) affected by the transplant destination (Mg, Cd, Fe, and Pb) was similar for Mg, Cd, and Fe (i.e. lowest concentration at PCO site, similar concentrations for the other two) and was just the opposite for Pb (i.e. highest concentration at PCO site, similar concentrations for the other two).

Because mayflies used in the transplant study were collected from two locations (HPO and PCO), it was possible to examine the effects of both source and destination on the final cation concentrations in the insects. For Zn, Na, and Cd, only their source was an important predictor ($p < 0.05$) of final concentrations. These three cations showed two patterns (Fig. 5). There were higher Na concentrations found in mayflies collected from PCO (pH 5.6) relative to those from HPO (pH 6.2, across all destinations), but higher Zn and Cd concentrations found in samples collected from HPO relative to those from PCO (again, across all destinations). In four cations (Al, Pb, Fe, and Mn), only a destination effect was observed; the site where the insects were collected did not significantly ($p > 0.05$) affect their final concentrations. The general pattern for these metals, allowing for some statistically imperceptible "noise," was high concentrations in those insects transplanted to HPO (pH 6.2) relative to samples transplanted to PCI (pH 4.2, Fig. 5). For Ca and Mg, the pattern of differences among concentrations in insects placed in the various destinations depended on where the insects had originally been collected (i.e. there was an interaction between source and destination). For both of these cations, mayflies from HPO that were replaced in HPO had the highest final concentration, followed by those transplanted to PCO, and finally PCI (Fig. 5). For samples collected from PCO, there were no differences in either Ca or Mg levels among mayflies transplanted to the three sites (Fig. 5).

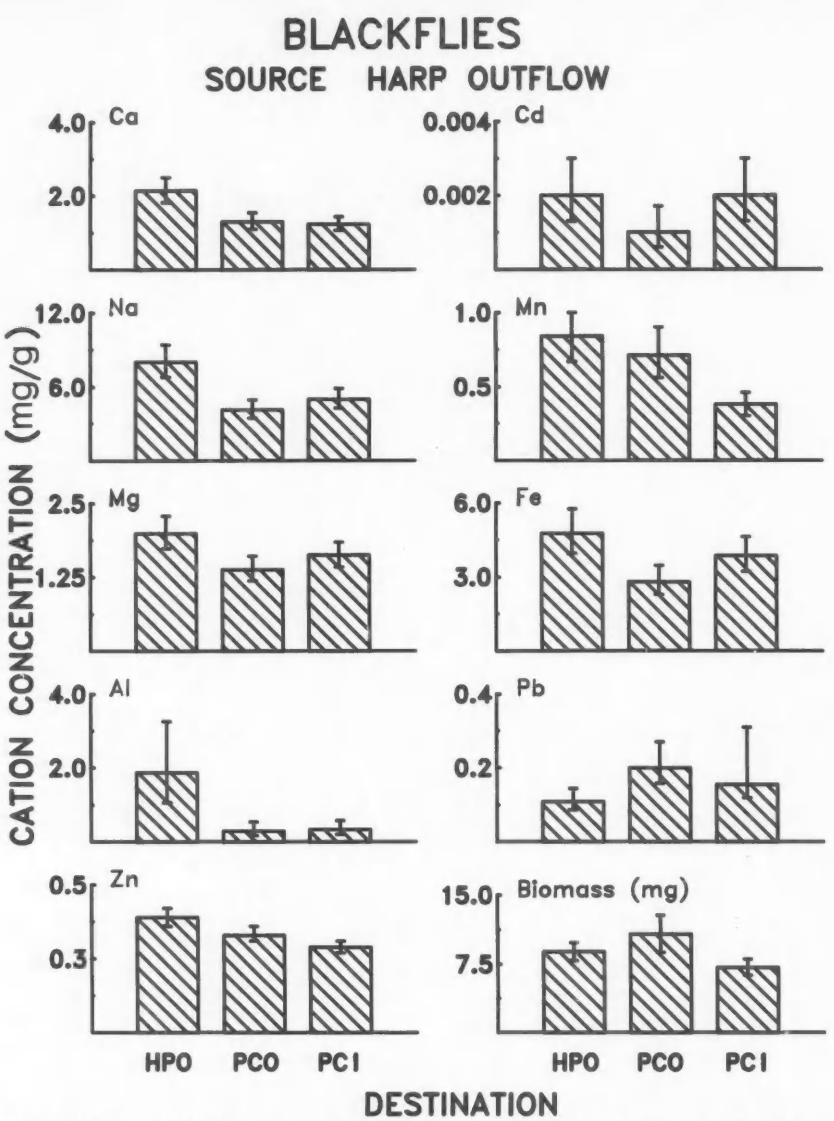

FIG. 4. Mean (±SE) chemical concentration and biomass of blackflies collected from Harp Outflow (HPO) and transplanted to HPO, Plastic Outflow (PCO), and Plastic Outflow No. 1 (PCI) destinations.

Adsorption and Absorption of Al

The predicted 96-h mean desorption of surface-associated Al relative to whole-body concentration in *L. cupida* at pH 4.1 was 3.3% for the controls (groups 1, 2, and 3) and 5.6% for the experimental groups (groups 4–8) (Table 2). The percent of mean whole-body Al concentration in gut contents was 65% (Table 3), leaving 29.4–31.7% absorbed in body tissues.

For *P. fuscum/mixtum* the predicted mean desorption of surface-associated Al relative to whole-body concentration at pH 4.1 was 1.6% for a group of organisms not used in the field transplants (control groups 1 and 2) and 1.6% for the experimental transplant groups (3 and 4) (Table 2). The percent of mean whole-body Al concentration in gut contents was 42% (Table 3), leaving 56.4% absorbed in body tissues.

Discussion

Leptophlebia cupida and *P. fuscum/mixtum* survived most short-term exposures to pH levels as low as 4.1 and the concomitant elevated metal concentrations in the water prior to and during snowmelt. Our original hypothesis that there would be reduced survival under low pH conditions was based on research showing that mayflies and blackflies are sensitive to high H^+ concentration (Hall et al. 1980). The experimental organisms were well over half grown during our exposure period and would have emerged from the water in May and June. Therefore, we feel that size of the organisms used in the experiments was important for tolerance. Havas (1981) reported that aquatic invertebrates can tolerate a lower pH when the organisms are large (pH ~4) than when they are small (pH ~5). Likewise,

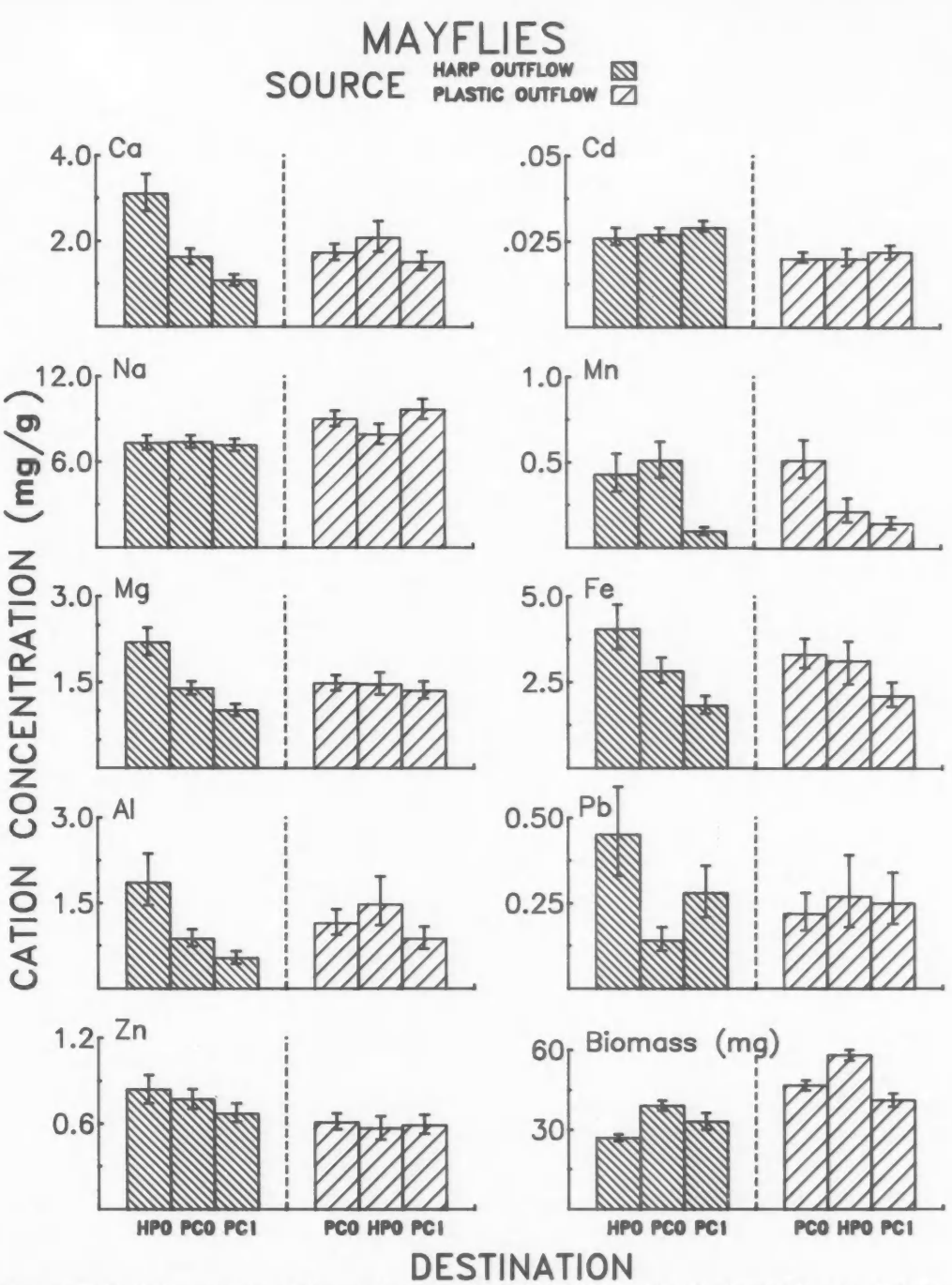

FIG. 5. Mean (± SE) chemical concentration and biomass of mayflies collected from the source (Harp Outflow (HPO) and Plastic Outflow (PCO)) and transplanted to HPO, PCO, and Plastic Outflow No. 1 (PCI) destinations.

Allard and Moreau (1987) concluded that small Leptophlebiid mayflies were eliminated at pH 4.0 but larger individuals survived.

A second possibility for survival at low pH was that the concentrations of cations (Ca, Mg, and Na) in the water were high enough to counteract the pH-Al toxicity (Leivestad et al. 1976). Zooplankton are known to survive longer at low pH (≤5.0) and high cation concentration, particularly Na and Ca, than at the same pH with low cation concentration (Havas 1981, 1985; Havas et al. 1984). More recent literature on the pH sensitivity

TABLE 2. Summary of predicted Al desorbed (mg/g) from the mayfly *L. cupida* and the blackflies *P. fuscum/mixtum* exposed to pH 4.1 lake water for 96 h. The indicated slopes and intercepts and RMS, r^2, F, and n are the parameters and regression diagnostics of linear regression equations of observed [Al] desorbed from organisms in test lake water on log time predicted from the indicated models. Percent [Al] lost was computed based on whole-body concentrations indicated in Table 3. Mayfly groups 1, 2, and 3 and blackfly groups 1 and 2 were not used in transplants and are considered controls.

Organism	Group	Slope	Intercept	RMS	r^2	F	n	Predicted readily desorbable [Al]	[Al] desorbed / whole-body [Al] × 100
Mayfly	1	0.029	−0.016	3.0×10^{-3}	0.89	48	8	0.14 ± 0.55	3.4
	2	0.024	−0.003	9.45×10^{-4}	0.99	434	7	0.13 ± 0.16	3.2
	3	0.027	−0.022	1.37×10^{-3}	0.99	2248	6	0.13 ± 0.18	3.2
								$\bar{x} = 0.13$	$\bar{x} = 3.3$
	4	0.043	−0.004	6.78×10^{-3}	0.88	44	6	0.20 ± 0.28	4.9
	5	0.065	−0.05	4.88×10^{-3}	0.91	29	5	0.31 ± 0.48	7.6
	6	0.041	−0.03	1.98×10^{-3}	0.99	195	5	0.20 ± 0.30	4.9
	7	0.048	−0.05	2.66×10^{-3}	0.86	19	5	0.22 ± 0.82	5.4
	8	0.041	−0.01	1.90×10^{-3}	0.96	78	5	0.21 ± 0.30	5.1
								$\bar{x} = 0.23$	$\bar{x} = 5.6$
Blackfly	1	0.007	0.014	3.0×10^{-3}	0.97	171	7	0.08 ± 0.11	1.6
	2	0.015	0.005	5.44×10^{-4}	0.99	327	7	0.08 ± 0.09	1.6
								$\bar{x} = 0.08$	$\bar{x} = 1.6$
	3	0.019	−0.015	1.84×10^{-3}	0.95	60	5	0.09 ± 0.14	1.8
	4	0.015	−0.012	2.26×10^{-3}	0.98	1.4	5	0.07 ± 0.12	1.4
								$\bar{x} = 0.08$	$\bar{x} = 1.6$

TABLE 3. Comparison of the amount of whole-body Al (mg/g, mean ± SE) in *L. cupida* and *P. fuxcum/mixtum* before and after (96 h) gut clearing.

Organism	No. of individuals per treatment × no. of treatments	Before evacuation	After evacuation	Decrease (%)
L. cupida	20 × 6	4.1 ± 0.22	1.45 ± 0.14	65
P. fuscum/mixum	30 × 6	5.0 ± 0.05	2.88 ± 0.02	42

of Leptophlebiid species combined with our results suggest that this group is sensitive in the pH 4.0–4.2 range and below. Allard and Moreau (1987) reported that individuals within the family Leptophlebiidae were eliminated during exposures to streamwater of pH 4.0. Rowe (1987) experimentally observed no mortality at pH 4.5 with winter populations of *L. cupida* but 30% mortality during short-term (4–8 d) pH depressions of 3.5.

The significant decrease in whole-body Ca concentration in both blackflies and mayflies and Na concentration in blackflies may indicate a change in ionoregulation with decreased pH. However, no cause-and-effect statement about reduction in Ca and mortality can be made, since little mortality was demonstrated. Havas and Hutchinson (1982) similarly found no correlation between mortality and reduced Ca concentrations at low pH (<4.5) for a caddisfly (*Limnephilus pallens*) and a midge (*Orthocladius consolerinus*). Furthermore, in laboratory experiments, Rowe (1987) reported no loss of Ca for two mayfly species (*L. cupida* and *Stenonema femoratum*) that had 30% mortality when exposed to pH 3.5 for 8 d, and Lechleitner et al. (1985) reported no loss in Ca for the stonefly *Pteronarcys dorsata* at pH 3.0. However, changes in the regulation of Ca at low pH have been reported for crayfish (Hollett et al. 1986; Wood and Rogano 1986) and cladocerans (Havas et al. 1984).

Loss of Na has been reported to occur in other insect species (Vangenechten et al. 1979; Vangenechten and Vanderborght 1980; Havas and Hutchinson 1982; Lechleitner et al. 1985) but only at pH levels ≤3.5. In laboratory experiments conducted on *L. cupida*, Rowe (1987) similarly showed no loss of Na at pH 4.5 but a significant decrease at pH 3.5. Compensatory mechanisms for cation loss at low pH in those organisms with little or no mortality are then internal replacement of cations from body tissues in aquatic Hemiptera (Vangenechten 1983), increased molting in mayflies, and an increase in the number of chloride cells in *L. cupida* (Rowe 1987).

Our second hypothesis, that metal accumulation would increase whole-body concentrations at lower pH levels, was likewise not supported by our data. This prediction was based on reports that concentrations of Al, Zn, Mn, and Fe are increased in surface waters during snowmelt as a result of mobilization from the edaphic environment to the aquatic phase during acidification (Johnson et al. 1969; Dickson 1978; Cronon and Schofield 1979; Driscoll et al. 1980; Baker and Schofield 1982) and on observations by Havas (1986) that Al accumulates on the respiratory structures of invertebrates. In our experiment, however, after 4 d of exposure to at least one lower pH level (~4.2), *L. cupida* and *P. fuscum/mixtum* had lower, not higher, whole-body concentrations of Al, Zn, Mn, and Fe. Similar results have been reported for algae (Campbell and Stokes 1985), stream bryophytes (Caines et al. 1985), crayfish (Malley et al. 1987), *Daphnia* (Havas 1985), unionid clams (Servos et al. 1987), a caddisfly (Otto and Svensson 1983), and a chironomid (Young and Harvey 1988).

For five of the nine cations (Zn, Ca, Mg, Na, and Cd), there were either source effects (Zn, Na, and Cd) or interactions between source and destination effects (Ca and Mg) on final concentrations found in the mayflies. These locational effects

may indicate a permanent or at least long-term environmentally induced influence on cation accumulation in either the egg or early instar stages. Alternatively, the two populations may differ genetically. If the latter hypothesis is true, then the HPO and PCO populations may represent different strains of *L. cupida* which have responded to the different selection pressures presented by streams with different pH and cation regimes.

In general, at pH levels ranging from 6.5 to 4.3, whole-body trace metal concentrations in mayflies and blackflies support the biotic response patterns proposed by Campbell and Stokes (1985) for bacteria, phytoplankton, and fish. Metals that display a Type I behaviour are those that demonstrate a decrease in biological response at low pH. We show in this paper that Al, Zn, Mn, and Fe (but not Cd) follow the Type I behaviour. Type II behaviour is characteristic of those metals with increased concentration in organisms at low pH. Although the results are only suggestive, we observed Type II behaviour for Pb in the blackfly transplants.

Trends in the data support the hypothesis that metal adsorption increases at lower pH levels. The percent of whole-body Al concentration in the adsorbed phase increased 70% (from 3.3 to 5.6% of whole-body concentration) for the transplanted (versus control) mayflies. Thus, a small increase in the amount of Al adsorbed took place simultaneously with a decrease in whole-body concentration. Havas (1986) determined that Al accumulated on the respiratory structures of insects. In this study, Al accumulated on mayflies (with external gills) and not on blackflies (with no external respiratory structures) but the significance of this small adsorbed fraction is unknown.

The percentage of whole-body Al concentration that was absorbed in *L. cupida* was smaller (29–32%) compared with gut contents (65%). The whole-body Al concentration of the organisms, and of other metals as well, therefore, may not have toxicological and/or physiological relevance (Smock 1983a). The benthic insects in this study appear to be more tolerant to elevated acidity and metals in water than many fish (Harvey and Lee 1982), amphibians (Clark and Hall 1985), and crustaceans (Stephenson and Mackie 1986). A major functional role of these dominant benthic organisms in Muskoka–Haliburton may be the transfer of metals from sediments to higher trophic levels either in the water (Havas and Jaworski 1986; Mengie 1980; G. Kranzberg, University of Toronto, Toronto, Ont., pers. comm.) or to the terrestrial environment via emergence (Nyholm 1981; Eriksson 1984; Ormerod et al. 1986; Herrmann 1987; Young and Harvey 1988).

Acknowledgements

Support for this research was provided by the Ontario Ministry of the Environment as part of the Acid Precipitation in Ontario Study. Many members of the staff at the Dorset Research Centre assisted with various aspects of this study. In particular, we thank K. Clark, J. Jones, and L. Scott for their assistance in the field. P. Dillon, D. Malley, G. Mierle, N. Yan, and A. Zimmerman made helpful comments on earlier drafts of this manuscript.

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Contaminant Effects on Ovarian Development in English Sole (*Parophrys vetulus*) from Puget Sound, Washington

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Johnson, L. L., E. Casillas, T. K. Collier, B. B. McCain, and U. Varanasi. 1988. Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington. Can. J. Fish. Aquat. Sci. 45: 2133–2146.

In a study evaluating the effects of exposure to xenobiotic compounds on ovarian development in English sole (*Parophrys vetulus*), prespawning females were sampled from four sites in Puget Sound, Washington, during the 1986 and 1987 spawning seasons. Two sampling sites had high concentrations of xenobiotic compounds in the sediment, while the other sites were less contaminated. The following factors associated with ovarian maturation were measured: ovarian developmental stage, ovarian atresia, gonadosomatic index, plasma estradiol, and plasma vitellogenin as estimated from alkali-labile phosphorus. Contaminant exposure was assessed by measuring concentrations of fluorescent aromatic compounds in the bile, hepatic aryl hydrocarbon hydroxylase (AHH) activity, and hepatic polychlorinated biphenyl levels, and liver tissue was examined histologically for the presence of suspected toxicopathic lesions. Female English sole from the heavily contaminated sites were significantly less likely to undergo gonadal recrudescence and had lower mean levels of plasma estradiol than females from the less contaminated sites. The risk of inhibited gonadal recrudescence was significantly increased in sole with elevated hepatic AHH activity, and AHH activity was also significantly negatively correlated with plasma estradiol level. These findings suggest that contaminant exposure may interfere with ovarian development in female English sole.

Dans le cadre de l'évaluation des effets sur le développement ovarien de la sole anglaise (*Parophrys vetulus*) de l'exposition à des composés xénobiotiques, des femelles au stade préfrai ont été prélevées en quatre endroits du détroit Puget (Washington) au cours des saisons de frai de 1986 et 1987. Les sédiments de deux des sites de prélèvements présentaient des concentrations élevées de composés xénobiotiques, ceux des deux autres sites étant moins contaminés. Les facteurs liés à la maturation ovarienne mesurées ont été : l'étape de développement ovarien, l'atrésie ovarienne, l'indice gonadosomatique, la teneur en estradiol plasmatique et la teneur en vitellogénine plasmatique estimée à partir du phosphore labile en milieu alcalin. L'exposition aux contaminants a été déterminée par la mesure des concentrations des composés aromatiques fluorescents présents dans la bile, de l'activité de l'aryl-hydroxylase des hydrocarbures (AHH) du foie et des teneurs en biphényles polychlorés du foie et par un examen histologique des tissus hépatiques pour la recherche de lésions toxicopathiques. Les soles anglaises femelles des sites fortement contaminés présentaient, de façon significative, un rétablissement gonadique moins important et des teneurs moyennes en estradiol plasmatique inférieures comparativement aux femelles des sites moins contaminés. Le risque d'une inhibition du rétablissement gonadique était significativement accru chez les soles présentant une activité élevée de l'AHH hépatique et l'activité de cet enzyme était aussi, de façon significative, négativement corrélée avec la teneur en estradiol plasmatique. Ces faits portent à croire que l'exposition aux contaminants peut nuire au développement ovarien chez les femelles de la sole anglaise.

Received April 25, 1988
Accepted August 29, 1988
(J9705)

Reçu le 25 avril 1988
Accepté le 29 août 1988

Exposure to xenobiotic compounds is believed to have a detrimental effect on ovarian development and spawning in teleost fish. A number of laboratory studies have been conducted to assess the impact of exposure to toxicants on ovarian development. Among the effects observed are inhibition of oocyte development and maturation, increased follicular atresia of both yolked and previtellogenic oocytes, abnormal yolk deposition and yolk formation within oocytes, and abnormal egg maturation and egg production (see Lam (1983) and Susani (1986) for reviews). However, relatively few field studies have been conducted to determine whether detrimental effects on ovarian development are found in natural marine fish populations exposed to environmental contaminants. Those studies that have been conducted suggest that under certain conditions, reproductive impairment may occur. Stott et al. (1983), for example, found altered ovarian development in plaice (*Pleuronectes platessa*) exposed to crude oil as a result of the Amoco Cadiz oil spill near Brittany, France. Further, Cross and Hose (1986) reported increased atresia of early stage oocytes in white croaker (*Genyonemus lineatus*) from contaminated sites near Los Angeles, California, while Slooff and DeZwart (1983) reported decreased gonadosomatic indices in bream (*Abramis brama*) from sites in the Rhine river with high levels of sediment-associated organochlorine compounds and aromatic hydrocarbons. In other studies, however, contaminant exposure appeared to have no clear effect on reproductive activity (Stott et al. 1980, 1981; Cross et al. 1984).

The objective of this study was to determine whether contaminant exposure was associated with altered ovarian development in English sole (*Parophrys vetulus*) from Puget Sound,

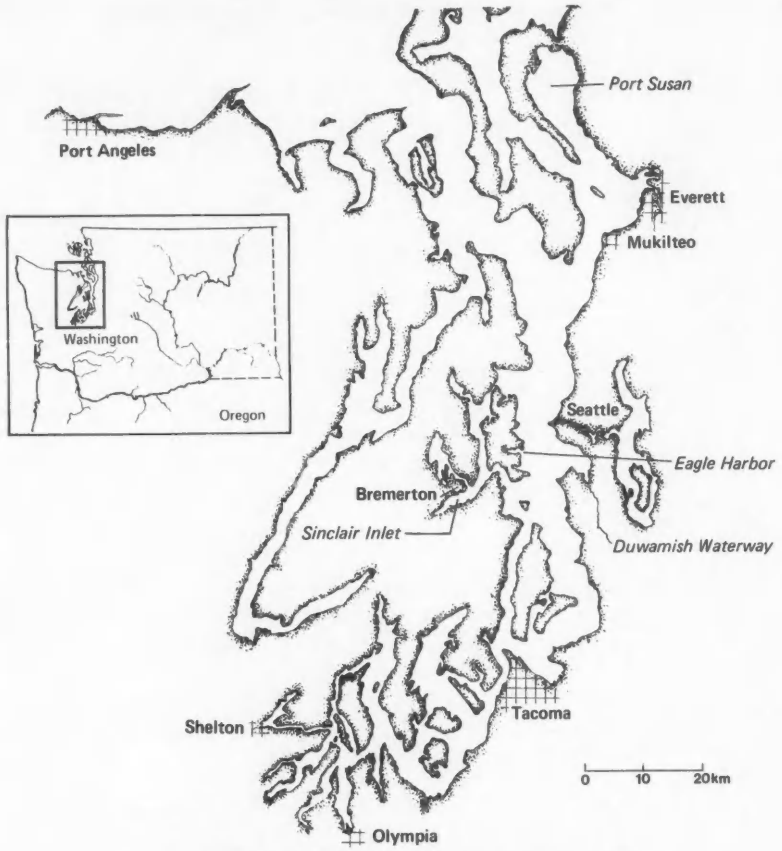

FIG. 1. Locations of sampling sites in Puget Sound, Washington.

Washington. English sole was chosen as the experimental animal because individuals experiencing a range of exposure levels could be obtained. In addition, this species exhibits high prevalences of suspected toxicopathic liver lesions (Malins et al. 1984, 1985), so their effect on vitellogenesis and ovarian development could be examined. The English sole were captured from four sites located in Eagle Harbor, Sinclair Inlet, Port Susan, and the Duwamish Waterway. The Duwamish Waterway and Eagle Harbor are both heavily contaminated; sediment aromatic hydrocarbon (AH) and polychlorinated biphenyl (PCB) concentrations are both high in the Duwamish Waterway, whereas in Eagle Harbor sediment AHs are extremely high but PCB concentrations are low (Malins et al. 1984, 1985). The site in Sinclair Inlet had relatively low levels of AHs and moderate levels of PCBs in the sediment, while the site in Port Susan was minimally contaminated (Malins et al. 1984). Contaminant exposure levels in individual fish were assessed by measuring levels of fluorescent aromatic hydrocarbons (FACs) in the bile, PCB levels in the liver, and hepatic aryl hydrocarbon hydroxylase (AHH) activity. Both AHs and PCBs have been associated with reproductive failure in fish in controlled laboratory exposures (Chen and Sonstegard 1984; Freeman et al. 1982; Hedke and Puglisi 1980; Payne et al. 1978; Rowe et al. 1983). AHH activity has been shown to be indicative of contaminant exposure in marine species (Collier and Varanasi 1987; Payne et al. 1987) and a relationship with reproductive dysfunction has been suggested in other studies (Spies et al. 1984, 1985).

Materials and Methods

Fish Capture and Collection of Samples

Resident female English sole were collected by otter trawl from Eagle Harbor, Sinclair Inlet, Port Susan, and the Duwamish Waterway in Puget Sound, Washington (Fig. 1). Sampling was conducted during the winters of 1985–86 and 1986–87 from November through January, the period in which vitellogenesis normally occurs in this species, but before substantial migration to spawning areas has taken place. Ten to 30 adult females (length ≥300 mm) were randomly selected for necropsy from each site at each sampling time. Females smaller than 300 mm were not included in the study to insure that all sampled females were large enough and old enough to have reached sexual maturity, which in female English sole occurs at approximately 260 mm or 3 yr of age (Garrison and Miller 1982). All sites were sampled within a 10-d period and equal numbers of fish were collected from each site during each set of samplings.

Sole were individually weighed and measured, otoliths were collected, and a 1-mL blood sample was taken with a heparinized syringe. Blood samples were subsequently

centrifuged at 800 × *g* and the plasma stored at −20°C for measurement of plasma, estradiol, and alkali-labile phosphorus (ALP) concentrations. Ovaries were weighed and samples were taken for histological examination. Livers were preserved in Deitrichs' fixative (Gray 1954), while ovaries were preserved in Davidsons' fixative (Mahoney 1973). A portion of the liver was also frozen immediately in liquid nitrogen and stored at −70°C for later determination of AHH activity. Additionally, tissue samples from the liver and ovary were collected and stored at −20°C for later determination of PCB concentrations. Bile was collected and stored at −20°C for measurement of FACs. Gonadosomatic index (GSI) was calculated according to the following formula:

$$\text{GSI} = (\text{ovary weight (g)}/\text{gutted body weight (g)}) \times 100.$$

Because low body weight may be associated with suppressed ovarian development in adult female fish (Burton and Idler 1987), a condition factor was determined for all sampled animals so the influence of emaciation on ovarian development could be distinguished from any potential effects of contaminant exposure. Condition factor was calculated according to the following formula:

$$\text{Condition factor} = \text{gutted body weight (g)}/\text{length}^3\ \text{(cm)}.$$

Analyses of Tissues and Fluids

Tissues collected for histology were embedded in paraffin, sectioned, stained with hematoxylin and eosin (Luna 1968), and examined microscopically. Hepatic lesions were classified according to the criteria outlined in Myers et al. (1987) and then grouped into the following categories: neoplasms (hepatocellular carcinoma, cholangiocellular carcinoma, adenoma, and cholangioma), foci of cellular alteration (eosinophilic foci, basophilic foci, clear cell foci), specific degenerative necrosis (nuclear pleomorphism and/or megalocytic hepatosis), and storage disorders (hemosiderosis and/or fatty change).

Ovaries were classified by developmental stage according to criteria modified from Wallace and Selman (1981) into the following categories: regressed (oocytes in the perinucleolar stage), previtellogenic (oocytes with cortical alveoli), and vitellogenic (oocytes with exogenous yolk deposition in the cytoplasm). Spawning or spawned out females were not observed because sampling was conducted before the onset of spawning.

Ovaries were also examined for follicular atresia, ovarian macrophage aggregates (MAs), and other inflammatory lesions associated with oocyte resorption, including lymphoid or macrophage infiltrates. Atretic follicles were classified according to the scheme described in Hunter and Macewicz (1985) as alpha yolked atretic follicles, alpha nonyolked atretic follicles, or beta, gamma, or delta follicles. Ovarian lesions were then divided into the following groups for statistical analysis: yolked atretic follicles (alpha stage), nonyolked atretic follicles (alpha stage), late stage atretic follicles (beta, delta, or gamma stage) and MAs, and idiopathic ovarian inflammatory lesions (i.e. lymphoid or macrophage infiltrates not associated with parasitic infection). Ovarian MAs were grouped with late-stage atretic follicles for subsequent analyses because of their resemblance to delta and gamma atretic follicles and because of the close association which we have observed between MAs and ovarian atresia in spawned out female English sole undergoing ovarian resorption. In addition, the actual proportion of yolked oocytes undergoing atresia in individual fish was determined by morphometric analysis for a subsample of females collected during the 1985–86 field season.

Hepatic PCB levels were determined from approximately 2-g samples, according to the method described by MacLeod et al. (1985) with modifications. Briefly, tissue samples were ground with 10 g of silica added to a column (270 × 23 mm) containing activated silica gel (Amicon Corp., Danvers, MA) and eluted with 50 mL of pentane — methylene chloride (90:10, V/V). The eluant was concentrated and exchanged with 1 mL of hexane prior to analysis by gas chromatography. FACs in the bile, which fluoresce at benzo[*a*]pyrene (BaP) or naphthalene (NPH) wavelengths, were measured according to the method of Krahn et al. (1986). Hepatic microsomes were prepared and hepatic AHH activity was measured using BaP as a substrate as described by Collier et al. (1986).

Plasma estradiol levels were determined by radioimmunoassay as described by Sower and Schreck (1982). Plasma vitellogenin levels were measured as ALP (DeVlaming et al. 1984). Phosphate was measured according to the method of Gamst and Try (1980).

Statistical Analyses

Hepatic and ovarian lesion prevalences were calculated for each sampling site, and the *G*-statistic (Sokal and Rohlf 1981) was used to determine whether prevalences in sole from minimally and heavily contaminated sites differed significantly. Because a series of probability tests was performed on the same data, the significance level chosen ($p \leq 0.05$) was divided by the number of tests performed to adjust for multiple comparisons (Miller 1981). As the one or more lesions category is not independent of the others, it was not counted as a separate analysis, so the 0.05 critical value was divided by 4 to give 0.0125 as the adjusted critical value.

Analysis of variance (Sokal and Rohlf 1981) was used to compare mean levels of plasma ALP, plasma estradiol, and GSI at the sampling sites. Analysis of variance was also used to determine the effect of ovarian maturation stage on AHH activity, so any variation in AHH activity associated with the reproductive cycle could be separated from changes in AHH activity associated with contaminant exposure.

Regression analysis (Sokal and Rohlf 1981) was used to examine relationships between variables associated with contaminant exposure (i.e. concentrations of FACs in bile, hepatic AHH activity, concentrations of PCBs in the liver, site of capture, and the presence of hepatic lesions) and variables associated with reproductive activity (i.e. GSI, plasma estradiol, and plasma ALP). The effects of fish length, condition factor, year of sampling, and ovarian developmental stage were taken into account in these analyses. For analyses involving the effect of site of capture, Sinclair Inlet was arbitrarily chosen as the reference site, and other sites were evaluated relative to Sinclair Inlet. Regression models were fitted using stepwise linear regression, with the significance level for entry of variables into the equation set at $p \leq 0.05$. Analyses were conducted using the SPSS-X statistical package (SPSS, Inc. 1983) and the Statview statistical package (Feldman and Gagnon 1986).

Logistic regression (Breslow and Day 1980) was used to assess the relationships between ovarian recrudescence and ovarian atresia and indicators of contaminant exposure (i.e. site of capture, hepatic AHH activity, bile FACs, hepatic PCBs, and the presence of hepatic lesions). This statistical method is similar to multiple regression, but is suitable for use with binom-

TABLE 1. Mean levels (± SD) of indicators of contaminant exposure in female English sole by site of capture. Bile for determination of fluorescent metabolites of aromatic compounds (FACs) was collected in 1986 and 1987, and aryl hydrocarbon hydroxylase (AHH) activity and liver PCB (polychlorinated biphenyl) data were collected in 1987. BaP wavelengths are compounds fluorescesing at wavelengths specific for BaP; includes 4–6 benzene ring AHs. NPH wavelengths are compounds fluorescesing at wavelengths specific for napthalene; includes 1–2 benzene ring AHs.

Sampling site	Biliary FACs: BaP wavelengths (ng/g)	Biliary FACs: NPH wavelengths (ng/g)	AHH (pmol·mg^{-1}·min^{-1})	Liver PCBs (μg/g wet wt)
Port Susan	36 ± 41[c,d] (n = 25)	15 208 ± 34 808[b,c,d] (n = 25)	221 ± 214[b,d] (n = 25)	0.52 ± 0.95[d] (n = 10)
Sinclair	155 ± 364[d] (n = 45)	56 313 ± 88 844[a,c,d] (n = 45)	72 ± 74[a,c,d] (n = 24)	3.21 ± 1.86[d] (n = 10)
Duwamish	218 ± 206[a,c] (n = 47)	87 052 ± 52 976[a,b] (n = 48)	492 ± 275[a,b,c] (n = 25)	14.2 ± 10.6[a,b,c] (n = 22)
Eagle Harbor	546 ± 407[a,b,d] (n = 47)	97 074 ± 64 164[a,b] (n = 47)	313 ± 242[b,d] (n = 25)	0.61 ± 0.22[d] (n = 10)

[a]Significantly different from Port Susan.
[b]Significantly different from Sinclair Inlet.
[c]Significantly different from Eagle Harbor.
[d]Significantly different from Duwamish Waterway (Fisher PL SD test, $p < 0.05$).

TABLE 2. Prevalences of hepatic lesions in female English sole captured from heavily contaminated and minimally contaminated sites in Puget Sound, WA. Sinclair Inlet, Eagle Harbor, and the Duwamish Waterway were sampled in both 1986 and 1987; Port Susan was sampled in 1987 only. Prevalences marked with an asterisk are significantly different from the prevalence for animals sampled at the two minimally contaminated sites combined (G-statistic, $p \leq 0.0125$).

	Neoplasms	Foci of cellular alteration	Specific degeneration/necrosis[a]	Storage disorders	One or more hepatic lesions[b]
Reference sites					
Port Susan (n = 50)	2 (1)	8 (4)	4 (2)	4 (2)	10 (5)
Sinclair Inlet (n = 99)	1 (1)	4 (4)	1 (1)	0 (0)	6 (6)
Contaminated sites					
Duwamish Waterway (n = 111)	36* (40)	59* (66)	32* (35)	40* (44)	77* (86)
Eagle Harbor (n = 93)	23* (21)	31* (29)	57* (53)	19* (18)	68* (63)
Reference Sites combined (n = 149)	1 (2)	5 (8)	2 (3)	1 (2)	7 (11)

[a]Nuclear pleomorphism and/or megalocytic hepatosis.
[b]Includes neoplasms, foci of cellular alteration, specific degeneration/necrosis, and storage disorders.

ially distributed outcome data (in this case, normal versus inhibited ovarian maturation, or the presence or absence of ovarian lesions). Fish length and condition factor were included in all analyses so the influence of these factors could be controlled. The influence of ovarian maturation stage was also taken into account in analyses examining contaminant impact on ovarian atresia. As in the multiple regression analyses, when the effect of site of capture was examined, Sinclair Inlet was arbitrarily chosen as the reference site and the effects of other sites were evaluated relative to Sinclair Inlet. The GLIM statistical package (Royal Statistical Society 1978) was employed to fit the logistic regression models using a procedure similar to stepwise regression with backward elimination of variables (Draper and Smith 1980). Variables were discarded from the models if their removal failed to produce a change in scaled deviance that was statistically significant ($p \leq 0.05$). For those conditions where a significant contaminant impact was found, predicted probabilities of the conditions (i.e. inhibited ovarian development or ovarian atresia) for individual fish, derived from the logistic regression model, were used to calculate predicted prevalences of the conditions at the sampling sites, and the predicted prevalences were compared with actual prevalences in the sampled populations using the z-test for equality of proportions (Fleiss 1981).

TABLE 3. Prevalences of females with inhibited ovarian development and mean values (±SD) of
and 1987 (nd = no data).

	Prevalences of females with inhibited ovarian development		Plasma estradiol (pg/mL)	
	1986	1987	1986	1987
Reference sites				
Port Susan	nd	20 (n = 50)	nd	3929 ± 3536[c,d,e] (n = 45)
Sinclair Inlet	17 (n = 46)	4 (n = 50)	1702 ± 1455 (n = 35)	5811 ± 3147[b,d,e] (n = 50)
Contaminated sites				
Duwamish	37[a] (n = 54)	36 (n = 47)	1232 ± 1358 (n = 44)	2531 ± 2313[b,c] (n = 43)
Eagle Harbor	34[a] (n = 47)	52[a] (n = 50)	1346 ± 1455 (n = 46)	1893 ± 2544[b,c] (n = 41)
Reference sites combined	17	12		
Significance	p = 0.0500	p = 0.0500	p = 0.3083	p = 0.0001

[a]Significantly different from prevalence for minimally contaminated sites (G-statistic, $p \leq 0.05$
[b]Significantly different from Port Susan.
[c]Significantly different from Sinclair Inlet.
[d]Significantly different from Eagle Harbor.
[e]Significantly different from Duwamish Waterway (Fisher PLSD, $p \leq 0.05$).

± SD) of plasma estradiol, plasma ALP (vitellogenin), and GSI by site for 1986

	Plasma ALP (μg/mL)		GSI	
	1986	1987	1986	1987
[b,d,e]	nd	27.02 ± 16.37 (n = 52)	nd	5.20 ± 3.90[c] (n = 45)
[b,d,e]	21.78 ± 11.0 (n = 45)	25.78 ± 10.88 (n = 49)	6.65 ± 4.82[c] (n = 45)	7.71 ± 8.62[b,d,e] (n = 50)
[c]	18.67 ± 13.81 (n = 44)	23.31 ± 18.39 (n = 49)	4.49 ± 3.72[c] (n = 54)	4.89 ± 3.93[c] (n = 48)
[c]	18.90 ± 13.62 (n = 48)	23.64 ± 21.20 (n = 50)	5.05 ± 4.29 (n = 48)	3.84 ± 4.17[c] (n = 50)
	p = 0.4425	p = 0.6439	p = 0.0394	p = 0.0057

≤ 0.05).

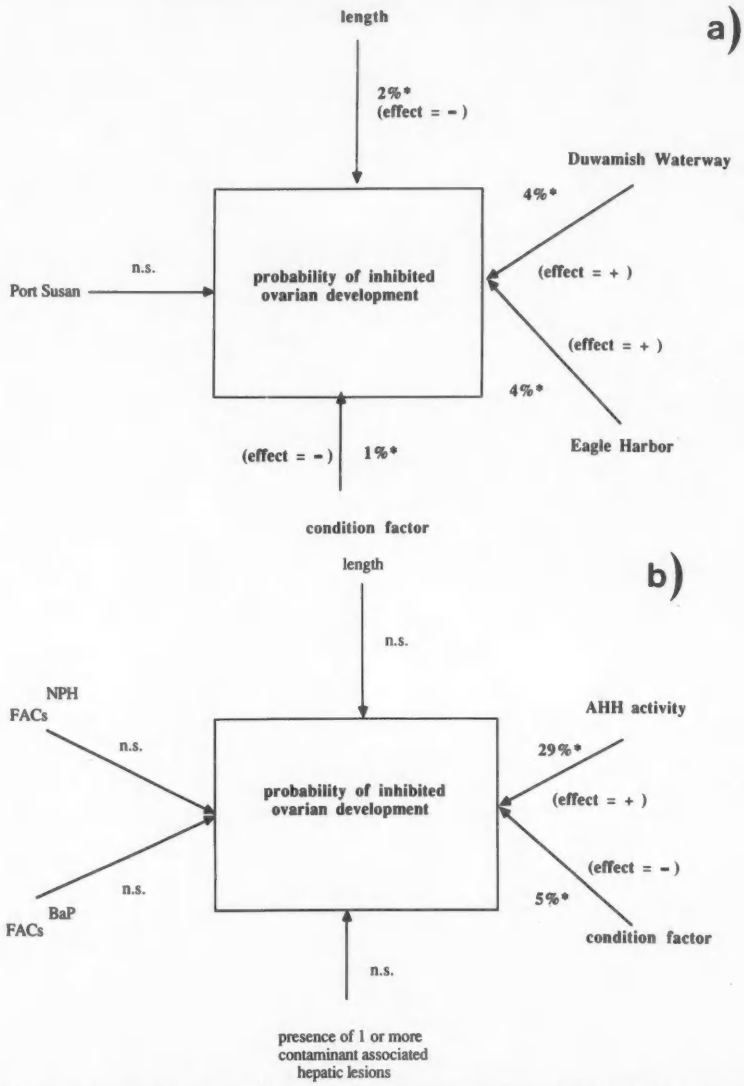

FIG. 2. (a) Predictive model evaluating the effect of site of capture on the probability of inhibited ovarian development in English sole while adjusting for the influence of fish size and condition factor. The model (Eagle Harbor + Duwamish Waterway; length and condition factor) accounts for 11% of the observed variation in the occurrence of arrested ovarian development (% = % of variation explained; n.s. = effect not significant at $p \leq 0.05$; asterisk = effect significant at $p \leq 0.05$). (b) Predictive model relating indicators of contaminant exposure to the probability of arrested ovarian development in English sole from Puget Sound. The model (AHH activity − condition factor) accounts for 34% of the observed variation in the occurrence of arrested ovarian development (% = % of variation explained; n.s. = effect not significant at $p \leq 0.05$; asterisk = effect significant at $p \leq 0.05$).

Results

Indicators of Contaminant Exposure in Sampled Animals

Sampled fish displayed a wide range of contaminant concentrations, but levels were generally highest in English sole captured in the Duwamish Waterway and at Eagle Harbor (Table 1). Concentrations of FACs in the bile of English sole from Eagle Harbor were significantly higher than in fish from any of the other sites sampled, while the concentrations of FACs in the bile of English sole from the Duwamish Waterway were significantly higher than in fish captured at Port Susan or Sinclair Inlet. PCB levels in the liver were significantly higher in fish from the Duwamish Waterway than in fish from the other sites. Hepatic AHH activity tended to be higher in fish from the Duwamish Waterway and Eagle Harbor than in fish from the other sites. Levels of biliary FACs and hepatic PCBs were significantly positively correlated ($p \leq 0.05$) with AHH activity. For FAC^{NPH} versus AHH (log-transformed values), $r = 0.238$ ($n = 98$); for FAC^{BaP} versus AHH (log-transformed values), r

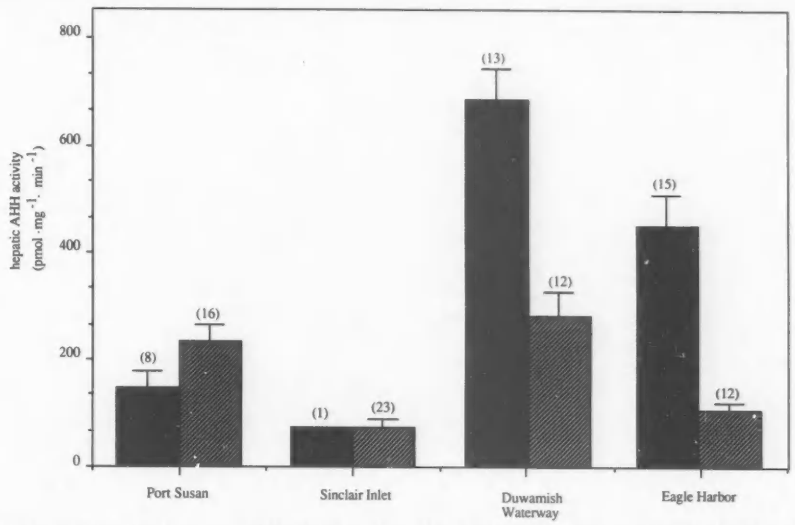

FIG. 3. Relationship between AHH activity and ovarian developmental stage in female English sole from Port Susan, Sinclair Inlet, Eagle Harbor, and the Duwamish Waterway. An asterisk indicates that stage means at that site are significantly different (ANOVA, $p \leq 0.05$). Numbers of animals examined are noted in parentheses. ■ = regressed; ▨ = vitellogenic.

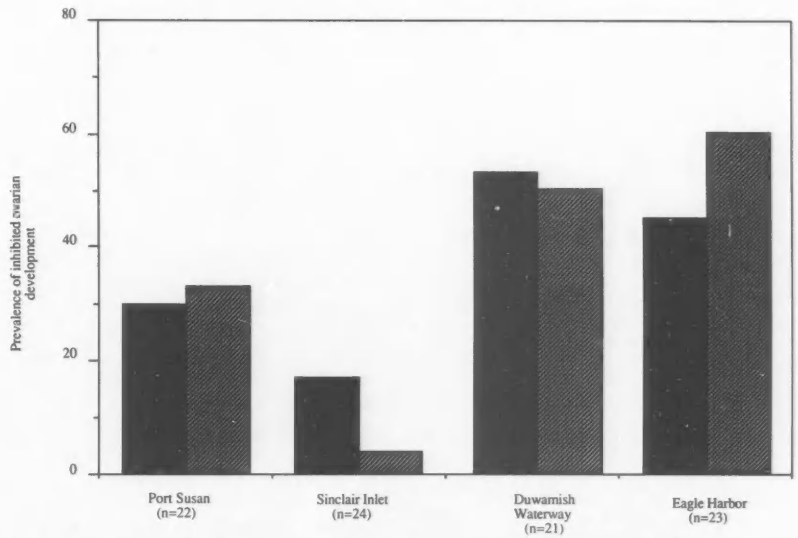

FIG. 4. Observed prevalences (percent affected) of inhibited ovarian development in English sole from Puget Sound versus prevalences predicted from GLIM model. ■ = predicted; ▨ = observed.

= 0.228 (n = 98); for hepatic PCBs versus AHH, r = 0.352 (n = 49).

Significantly higher prevalences of all categories of hepatic lesions were found in sole from the Duwamish Waterway and Eagle Harbor than in sole from Port Susan and Sinclair Inlet (Table 2). The proportion of females with one or more lesions was 10% or less at Port Susan and Sinclair Inlet, but in the Duwamish Waterway and at Eagle Harbor, respectively, 77 and 68% of sampled females had lesions. The most common lesion in English sole from Eagle Harbor was specific degeneration/necrosis, while the most common lesions in English sole from the Duwamish Waterway were foci of cellular alteration.

Ovarian Development

Assessment of ovarian development in English sole showed that although gonadal recrudescence was well underway in the majority of females sampled, a substantial number of adult females had not entered vitellogenesis. Ovarian development in these females had not proceeded beyond the perinucleolar or early previtellogenic stage. The prevalence of this type of inhibited ovarian development was significantly higher in sole from Eagle Harbor and the Duwamish Waterway than in sole from Sinclair Inlet and Port Susan, in both the 1986 and 1987 spawning seasons (Table 3). These differences in the proportions of maturing females were not due to between-site variation in fish

TABLE 4. Results of multiple regression analysis examining effects of site of capture, ovarian maturation stage, length, condition factor, year sampled, and hepatic lesions on GSI, plasma ALP (vitellogenin), and plasma estradiol. Only data from 1987 were included in the analyses because AHH activity was not available for 1986 sampling. ns = not significant.

Dependent variable	df	*F*-test	R^2	Independent variables							
				DW[a]	EH[a]	PS[a]	Ovarian maturation stage	Length	Condition factor	Sampled in 1986	One or more hepatic lesions[b]
GSI	336	61.08 $p = 0.0001$	0.355	ns	ns	ns	(+) $p = 0.0001$	(+) $p = 0.0001$	(+) $p = 0.0012$	ns	ns
ALP (vitellogenin)	333	109.43 $p = 0.0001$	0.398	ns	ns	ns	(+) $p = 0.0001$	ns	ns	(−) $p = 0.0002$	ns
Estradiol	299	56.84 $p = 0.0001$	0.435	(−) $p = 0.0006$	(−) $p = 0.0003$	ns	(+) $p = 0.0001$	ns	ns	(−) $p = 0.0001$	ns

[a]DW = Duwamish Waterway, EH = Eagle Harbor, PS = Port Susan. Sinclair Inlet is not included because it is used as a reference; the effect of capture at the other three sites is evaluated relative to Sinclair Inlet.

[b]Hepatic lesions include neoplasms, foci of cellular alteration, specific degeneration/necrosis (nuclear pleomorphism and/or megalocytic hepatosis), and storage disorders (fatty change and/or hemosiderosis).

FIG. 5. Relationships between (a) plasma ALP (vitellogenin) level, (b) plasma estradiol level, (c) GSI (ovary wt(g)/gutted body wt(g)·100), and ovarian maturation stage.

size or condition factor; when the influence of fish size and condition factor on ovarian development were taken into account using logistic regression, the probability that a female would show inhibited ovarian development was still significantly higher ($p \leq 0.05$) in the Duwamish Waterway and at Eagle Harbor than at the other two sites (Fig. 2a).

Logistic regression analysis revealed that of the various indicators of contaminant exposure which we measured, AHH activity was the best predictor of whether or not normal ovarian development would occur (Fig. 2b). Sole with elevated hepatic AHH activity were significantly less likely to undergo gonadal recrudescence than other animals. It should be noted that in fish captured at the reference sites, hepatic AHH activities did not vary with ovarian maturation stage (ANOVA, $p \leq 0.05$), suggesting that AHH activity was not affected by the onset of vitellogenesis. However, in sole captured at the contaminated sites, AHH activities were significantly higher (ANOVA, $p < 0.05$) in nonvitellogenic sole than in vitellogenic sole (Fig. 3). When the influence of AHH activity was not considered in the model, concentrations of FACs in the bile which fluoresce at BaP wavelengths and the presence of contaminant-associated hepatic lesions both explained a significant proportion of the variation in the occurrence of inhibited ovarian development ($p \leq 0.05$). However, they did not explain a significant additional proportion of the variation when the effect of AHH activity was taken into account. Condition factor had a significant negative influence on the probability of inhibited ovarian development (Fig. 2b); however, hepatic PCB concentration, concentrations of

FACs in the bile which fluoresce at NPH wavelengths, and fish length did not explain a significant additional proportion of the variation. The predicted percentages of female English sole with inhibited ovarian development were not statistically different from those actually observed for any of the four sites sampled (Fig. 4, z-test for equality of proportions, $p \leq 0.05$).

Plasma Estradiol, ALP (Vitellogenin), and GSI

Female English sole from Eagle Harbor and the Duwamish Waterway had consistently lower plasma estradiol levels than female English sole from either Sinclair Inlet or Port Susan during both sampling seasons (Table 3). These differences were not great enough to be statistically significant in the 1986 sampling, but in the 1987 sampling they were significantly different (Table 3). GSI was significantly lower in fish from the Duwamish Waterway than in fish from Sinclair Inlet in 1986 and was significantly lower in fish from both the Duwamish Waterway and Eagle Harbor than in fish from Sinclair Inlet in 1987. Plasma ALP levels were slightly lower in sole from heavily contaminated sites than in sole from minimally contamined sites, but these differences were not statistically significant (Table 3).

Plasma estradiol, plasma ALP, and GSI were closely related to ovarian maturation stage (Fig. 5). Consequently, multiple regression analysis was used to determine the extent to which the observed differences in GSI, estradiol, and ALP in sole from heavily contaminated and minimally contaminated sites could be accounted for by the difference in the proportion of vitellogenic females at these sites. Length, condition factor, and year sampled were also simultaneously adjusted for in these analyses (Table 4). In the case of GSI, intersite variation could be accounted for by variation in ovarian maturity stage, length, and condition factor; all three of these variables were positively correlated with GSI (Table 4). Differences in plasma ALP levels could also be accounted for by variation in ovarian maturity stage. However, for plasma estradiol, significant intersite differences were still found when the effect of ovarian maturity stage was taken into account. Even in females of the same ovarian maturation stage, plasma estradiol levels were lower in fish from Eagle Harbor and the Duwamish Waterway than in fish from the other sites. Both plasma estradiol and plasma ALP levels were lower in fish sampled in 1986 than in fish sampled in 1987; this was probably due to slight modifications in the assay techniques used in the second year of sampling. The presence of hepatic lesions did not have a significant effect on GSI, plasma ALP, or plasma estradiol.

Multiple regression analysis was applied to the data to determine which measures of contaminant exposure were the best predictors of plasma estradiol, plasma ALP, and GSI. Both GSI and plasma ALP level were dependent only on the ovarian maturity stage of the animal sampled (Table 5). Plasma estradiol levels, however, were dependent on both the ovarian maturity stage of the sampled animal and its level of hepatic AHH activity (Table 5), meaning that even in English sole of the same developmental stage, those with elevated AHH activity had decreased plasma levels of estradiol.

Ovarian Lesions

Nonvitellogenic sole were significantly more likely to show oocyte atresia and associated ovarian lesions than those that had entered vitellogenesis (G-statistic, $p \leq 0.0125$); fully 96% of nonvitellogenic sole (Table 6) had one or more lesions associated with resorption compared with 59% of vitellogenic females (Table 7). The most common lesions in nonvitellogenic sole were atretic nonyolked oocytes and ovarian MAs or late-stage atretic follicles. The types of lesions exhibited by nonvitellogenic females at the reference sites were generally similar to those at the contaminated sites. However, the prevalences of ovarian inflammatory lesions and ovarian MAs and late-stage atretic follicles were significantly higher in sole from the Duwamish Waterway and Eagle Harbor than in sole from the reference sites, while the prevalence of atresia of nonyolked oocytes was significantly lower in sole from the Duwamish Waterway than in sole from the other sites (Table 6). Vitellogenic females from all sites also exhibited oocyte atresia, but there was no clear difference in the prevalence of atresia between contaminated and reference sites (Table 7). Moreover, the actual morphometrically determined percentage of yolked oocytes undergoing atresia was relatively low at all sites samples, and no significant differences were seen among the sites. The mean percentages of yolked oocytes undergoing atresia at the four sites were 6.8 ± 4.1 ($n = 12$) at Eagle Harbor, 8.4 ± 10.3 ($n = 14$) at Sinclair Inlet, 5.3 ± 4.7 ($n = 6$) at the Duwamish Waterway, and 6.9 ± 5.2 ($n = 16$) at Port Susan.

Logistic regression was used to examine the relationship between contaminant exposure and ovarian lesions (i.e. atresia

TABLE 5. Results of multiple regression analysis examining effects of hepatic AHH activity, concentrations of FACs in bile that fluoresce at BaP and NPH wavelengths, length, condition factor, and ovarian maturation stage on GSI, plasma ALP (vitellogenin), and plasma estradiol. Only data from 1987 were included in the analyses because AHH activity was not available for 1986 sampling.

				Independent variables						
Dependent variable	df	F-test	R^2	AHH	BaP	NPH	Ovarian maturation stage	Length	Condition factor	One or more hepatic lesions[a]
GSI	88	58.07 $p = 0.0001$	0.400	ns	ns	ns	(+) $p = 0.0001$	ns	ns	ns
ALP (vitellogenin)	87	67.02 $p = 0.0001$	0.438	ns	ns	ns	(+) $p = 0.0001$	ns	ns	ns
Estradiol	91	45.49 $p = 0.0001$	0.502	(−) $p = 0.0323$	ns	ns	(+) $p = 0.0001$	ns	ns	ns

[a]Hepatic lesions include neoplasms, foci of cellular alteration, specific degeneration/necrosis (nuclear pleomorphism and/or megalocytic hepatosis) and storage disorders (fatty change and/or hemosiderosis).

TABLE 6. Prevalences of ovarian lesions in nonvitellogenic female English sole. Sinclair Inlet, Eagle Harbor, and the Duwamish Waterway were sampled in both 1986 and 1987; Port Susan was sampled in 1987 only. Prevalences marked with an asterisk are significantly higher or lower than the prevalence for animals from the two minimally contaminated sites combined (G-statistic, $p \leq 0.0125$).

	Atresia of nonyolked oocytes	Ovarian inflammation	Ovarian MAs and/or late-stage atretic follicles	One or more ovarian lesions[a]
Reference sites				
Port Susan (n = 10)	90 (9/10)	10 (1/10)	10 (1/10)	90 (9/10)
Sinclair Inlet (n = 9)	56 (5/9)	0 (0/9)	33 (3/9)	78 (7/9)
Contaminated sites				
Duwamish Waterway (n = 36)	47* (18/36)	14* (5/38)	55* (21/38)	78 (30/38)
Eagle Harbor (n = 39)	64 (25/39)	21* (9/41)	54* (23/41)	92 (38/41)
Reference Sites combined (n = 19)	74 (14/19)	5 (1/19)	21 (4/19)	84 (16/19)
All sites Combined (n = 94)	61 (57/94)	15 (14/94)	48 (45/94)	85 (80/94)

[a]Includes atresia of nonyolked oocytes, ovarian inflammation, ovarian MAs, and late-stage atretic follicles.

of yolked oocytes, atresia of nonyolked oocytes, late-stage atretic follicles and MAs, inflammatory infiltrates, and the presence of one or more of any of the preceding lesions). It was found that while females that had not entered vitellogenesis were more likely to exhibit ovarian lesions than vitellogenic females, neither the presence of hepatic lesions nor elevated levels of any of the indicators of contaminant exposure which we measured were associated with an increased risk of any ovarian lesion, once the influence of ovarian maturation stage had been taken into account.

Discussion

The results of this study suggest that exposure to xenobiotic compounds in the environment may have a detrimental effect on the reproductive processes in wild English sole. Female sole captured at highly contaminated sites, or with elevated AHH activity, were less likely than other females to enter vitellogenesis and had depressed plasma levels of estradiol. These findings are consistent with several reports in the literature linking disrupted or inhibited ovarian development and altered steroid metabolism to contaminant exposure. In laboratory studies, disrupted gonadal maturation has been reported in conjunction with exposure to chlorinated and organophosphate pesticides (Eller 1981; Saxena and Mani 1985). Similar conditions have also been noted in natural populations. Stott et al. (1983), for example, found that female plaice collected at the site of the Amoco Cadiz oil spill showed disrupted ovarian maturation cycles, with follicles remaining in the regressed stage during the normal spawning period. Several workers have found alterations in steroid metabolism in conjunction with contaminant exposure. In Atlantic cod (*Gadus morhua*) (Freeman et al. 1982) the hormone synthesizing ability of the testis was impaired with dietary exposure to PCBs. Sivarajah et al. (1978) showed decreased androgen and estrogen levels in trout and carp after exposure to PCBs. Truscott et al. (1983) similarly observed decreases in testosterone in the blood of landlocked salmon (*Salmo salar sepago*) and winter flounder (*Pseudopleuronectes americanus*) exposed to crude oil. These findings tend to corroborate our observations in English sole.

Of the indicators of contaminant exposure which we measured, hepatic AHH activity was the best predictor of whether or not a female would undergo normal gonadal recrudescence, and in addition, this indicator was negatively correlated with plasma estradiol level. In combination with condition factor, hepatic AHH activity accounted for 34% of the observed variation in the occurrence of ovarian development. This still leaves a considerable amount of the variation unaccounted for by factors not measured in this study, implying that factors such as unmeasured contaminants, genetic variation, health, or seasonal fluctuations in the spawning cycle may be affecting gonadal recrudescence in English sole. Nevertheless, the similarity between predicted prevalences of inhibited ovarian development and actual prevalences observed suggests that AHH activity may be a useful indicator in field studies designed to monitor contaminant effects on ovarian development. One potential problem with using AHH as an indicator of contaminant exposure in gonadally maturing fish is the possibility that hepatic AHH activity may vary naturally with the reproductive cycle. There is some evidence that in fish species other than English

TABLE 7. Prevalences of ovarian lesions in vitellogenic female English sole. Sinclair Inlet, Eagle Harbor, and the Duwamish Waterway were sampled in both 1986 and 1987; Port Susan was sampled in 1987 only. Prevalences marked with an asterisk are significantly different from the prevalence for animals from the two minimally contaminated sites combined (G-statistic, $p \leq 0.0125$).

	Atresia of yolked oocytes	Atresia of nonyolked oocytes	Ovarian inflammation	Ovarian MAs and late-stage atretic follicles	One or more ovarian lesion[a]
Reference sites					
Port Susan (n = 43)	35 (15/43)	16 (7/43)	5 (2/43)	9 (4/43)	56 (24/43)
Sinclair Inlet (n = 88)	47 (41/88)	24 (21/88)	1 (1/88)	18 (16/88)	60 (53/88)
Contaminated sites					
Duwamish Waterway (n = 71)	24* (17/71)	8* (6/71)	6 (4/71)	37* (26/71)	56 (40/71)
Eagle Harbor (n = 59)	53 (31/59)	25 (15/59)	3 (2/59)	14 (8/59)	68 (40/59)
Reference sites combined (n = 131)	43 (56/131)	21 (28/131)	2 (3/131)	15 (20/131)	59 (77/131)
All sites combined (n = 261)	40 (104/261)	19 (49/261)	3 (9/261)	21 (54/261)	60 (157/261)

[a]Includes atresia of nonyolked oocytes, ovarian inflammation, ovarian MAs, and late-stage atretic follicles.

sole, AHH activity declines with the onset of vitellogenesis (Walton et al. 1983; Lindstrom-Seppa 1985), which would limit its utility as an indicator of contaminant exposure during the reproductive season. In English sole, AHH activity does decline in actively spawning females (Collier et al. 1986). However, in this study, hepatic AHH activity did not change with the onset of vitellogenesis in females from the reference sites. Accordingly, the variations found in AHH activity in the current study can most probably be attributed to contaminant exposure.

Several investigators have found relationships between elevated AHH activity and various indicators of reproductive impairment. For example, increased activity of mixed function oxidases, including AHH, has been associated with decreased steroid levels in rainbow trout and carp (Sivarajah et al. 1978) and with decreased fertilization success in starry flounder (Spies et al. 1984, 1985). The exact role that AHH and other hepatic xenobiotic metabolizing enzymes may play in generating reproductive abnormalities is not clear. It has been suggested (Spies et al. 1984) that increased AHH activity itself may cause reproductive impairment by altering steroid metabolism and causing a decline in circulating steroid hormone levels. However, a causal link between AHH activity and reproductive dysfunction has not been established, and it is not clear that AHH plays an important role in steroid metabolism (Snowberger and Stegeman 1987). While we did see a significant negative correlation between hepatic AHH activity and plasma estradiol level in English sole, other related enzymes which are actively involved in steroid metabolism in fish (e.g. UDPglucuronosyltransferase) may also be affected by contaminant exposure (Hansson et al. 1980; Forlin and Hansson 1982) and could be having an impact on steroid metabolism and ovarian development in English sole. Studies are currently underway in our laboratories to investigate the effects of contaminant exposure on both the disposition of estradiol and the enzymes involved in steroid metabolism in English sole. Preliminary results show that pretreatment of juvenile English sole with xenobiotic compounds extracted from Duwamish Waterway sediment increases the excretion of [^{3}H]estradiol-17β, resulting in lower levels of estradiol in the plasma and higher levels in the bile compared with controls (Stein et al. 1988). Moreover, pretreatment of gravid female English sole with sediment extract decreased levels of endogenous estradiol. However, the roles that specific hepatic enzymes may play in altering estradiol metabolism have not yet been established.

Of the two classes of xenobiotic compounds, AHs and PCBs, whose effects on reproductive activity we examined, AHs, measured as FACs in the bile, appeared to be the most closely associated with inhibited ovarian development and depressed plasma estradiol. In bivariate analyses, significant negative relationships were found between levels of FACs in the bile and plasma estradiol and vitellogenin levels. Additionally, females with elevated levels of FACs in the bile were more likely than females with low levels of FACs in the bile to exhibit inhibited ovarian development. These relationships, however, may have been weaker than those found between AHH activity and inhibited ovarian development because bile FAC concentrations were more variable and may be affected by such factors as the feeding status of the sampled animals (Collier and Varanasi 1986; Krahn et al. 1986). This variability could make a clear relationship between bile FAC levels in individual fish and inhibited ovarian development more difficult to establish. Hepatic PCB concentrations, however, were not correlated with either estra-

diol or vitellogenin levels and were not associated with an increased risk of inhibited ovarian development even when the effect of AHH activity was not taken into account. Furthermore, although PCB concentrations were high in the livers of English sole sampled from the Duwamish Waterway, the prevalence of inhibited ovarian development at this site was no greater than the prevalence of inhibited ovarian development at Eagle Harbor, a site with high AH levels but no appreciable PCB contamination. It would be premature to draw any firm conclusions about the involvement of PCBs in inhibiting ovarian development at this point, however. Because no site was sampled in which PCB levels were high but AH levels were low, it is difficult to separate the effects of these chemicals on ovarian maturation, or to make accurate statements about any possible interactions. It is also important to recognize that contaminants other than those measured in this study could be involved in both the induction of AHH activity and the inhibition of ovarian development.

Hepatic lesions did not appear to have a direct impact on ovarian maturation. While it is plausible that severe liver degeneration could interfere with exogenous vitellogenesis, which could in turn lead to impaired oocyte development, we have little evidence to suggest that this is the case for the majority of females with hepatic lesions examined in this study. No significant differences were found in plasma ALP levels in English sole sampled from heavily contaminated and minimally contaminated sites, in spite of clear differences in hepatic lesion prevalences. Moreover, when the effects of other indicators of contaminant exposure were taken into account, the presence of hepatic lesions did not result in a significantly increased risk of inhibited ovarian development, as one would expect if the lesions had a direct effect on vitellogenesis.

Other variables associated with reproductive activity (i.e. atresia, GSI, and plasma vitellogenin as estimated from plasma ALP) were not as strongly affected by contaminant exposure as ovarian recrudescence and estradiol level. English sole from heavily contaminated sites did display reduced GSIs. However, in animals of the same maturity stage, GSI was similar at both heavily contaminated and minimally contaminated sites. This suggests that egg production is normal in female English sole that successfully enter vitellogenesis. To establish this conclusively, however, more detailed measurements of the actual fecundity of females in contaminated and uncontaminated sites would be necessary, particularly since contaminant effects on fecundity have been noted in other investigations (Cross and Hose 1986; Whipple et al. 1987). Similarly, plasma vitellogenin level did not appear to be affected by contaminant exposure. No significant differences in the plasma ALP levels of female English sole captured from heavily contaminated and minimally contaminated sites were found, either before or after the effect of ovarian maturation stage was taken into account. The results of laboratory studies investigating the effects of contaminant exposure on vitellogenesis, however, suggest that the impact of contaminants on plasma vitellogenin levels can be quite variable. Exposure to contaminants may decrease plasma levels of vitellogenin by reducing vitellogenin production (Chen and Sonstegard 1984), or increase plasma levels of vitellogenin by decreasing the uptake of vitellogenin by developing oocytes (Ruby et al. 1987). Both of these processes could have been occurring in the fish sampled in this study, making it difficult to detect any clear-cut effect of contaminant exposure on vitellogenin production. Additionally, it should be noted that the plasma ALP level is an indirect estimate of the level of vitellogenin in the plasma, and contaminant-associated effects on vitellogenin production may have occurred that were not detectable with this assay method.

No clear relationship was seen in this study between atresia and contaminant exposure. While a high proportion of vitellogenic females showed some signs of atresia, the actual percentage of oocytes affected was relatively low. This type of mild atresia is generally considered to be normal in teleost fish undergoing ovarian recrudescence (Guraya 1986). Female English sole from the Duwamish Waterway and Eagle Harbor were more likely than other sole to exhibit late stage atretic follicles, suggesting that resorption may begin earlier in the season or be a more chronic process in fish from these sites than in fish from Port Susan or Sinclair Inlet, but the impact that this may have on egg production is not clear. We also noted increased resorption of nonvitellogenic oocytes in English sole with inhibited ovarian development, which were found primarily at Eagle Harbor and in the Duwamish Waterway. In mammals, induced ovarian AHH activity and destruction of primordial oocytes have been shown to occur after exposure to aromatic hydrocarbons (Mattison and Nightingale 1980; Mattison et al. 1983). It is possible that the oocyte resorption observed in English sole in this study could also be related to AH exposure, especially since it has been demonstrated that AHs are taken up by the ovary of English sole and interact with gonadal macromolecules in the sole ovary in much the same way as they do in mammals (Varanasi et al. 1982). No relationship was found, however, between the level of contaminant exposure in the nonvitellogenic females examined and the probability that they would show oocyte atresia or related ovarian lesions. This could be because the majority of nonvitellogenic females came from sites with high levels of AHs in the sediment, so the range of exposure was not great enough to allow any clear relationship to be discerned.

In summary, our present results indicate that female English sole residing in contaminated areas may show reproductive impairment. Female sole from heavily contaminated sites are less likely than other female sole to undergo ovarian development, and have depressed levels of plasma estradiol. Both of these conditions are associated with elevated hepatic AHH activity. At this point, however, the impact which these reproductive anomalies may have on the abundance of English sole in Puget Sound is not clear. There is some evidence that populations of English sole in the Sound have been declining over the past years (Bargmann 1988), but the role that contaminant exposure might play in this decline is unknown. This is an important question that should be addressed in future studies.

Acknowledgements

We thank Bob Bunnell and Pat Manning for processing histological samples; Mark Myers, Paul Olson, and Linda Rhodes for their assistance in microscopic examination of liver and ovary tissues and diagnosis of lesions; Peggy Krahn and Leslie Moore for performing HPLC analyses of bile; Walt Dickoff, Penny Swanson, and Melinda Bernard for performing plasma estradiol assays; Ethel Blood for performing plasma alkali-labile phosphorus assays; Bill Gronlund, Dick Hughes, and Doug Weber for their assistance in capturing fish; and Mark Myers, John Stein, and Sin-Lam Chan for reviewing the manuscript. These studies were partially supported by the Office of Oceanography and Marine Assessment, National Ocean Services, NOAA.

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Evidence for Indirect Effects of Fish Predation on Maternal Lipid Investment in *Holopedium gibberum*

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Arts, M. T., and W. G. Sprules. 1988. Evidence for indirect effects of fish predation on maternal lipid investment in *Holopedium gibberum*. Can. J. Fish. Aquat. Sci. 45: 2147–2155.

We examined selected aspects of the reproductive dynamics of *Holopedium gibberum* in 25 lakes in Algonquin Park, Ontario. Using an index based on *Daphnia* size, we divided the lakes into two groups characterized as high and low fish predation. Overall, egg volumes were found to be a good predictor of energy reserves in stage 1 eggs of *Holopedium*. However, small eggs, which were associated with small females typical of the high predation lakes, had relatively less fat than large eggs, suggesting that fish predation indirectly reduces starvation resistance of *Holopedium* neonates. In addition, in high-fish lakes, mean adult body size, minimum size of ovigerous females, clutch size, and fat volumes of *Holopedium* were smaller than in lakes characterized as low fish predation. Maternal lipid investment (MLI) into eggs of *Holopedium* was compared between the two lake types. We found striking differences in the seasonal pattern of absolute MLI between the two lake groups, mostly mediated through differences in body size. The greatest difference in absolute MLI between the two lake types occurred in May; females from the low-predation lakes invested 4 times as much energy reserves into reproduction as compared with females from the high-predation lakes at this time. Relative MLI (energy reserves per microgram female weight), comparing only individuals which overlapped in size, was unaffected by lake type. Thus a constant proportion of an ovigerous female's weight is invested in reproduction with a seasonal component that matches standard algal abundance for many shield lakes, suggesting that relative MLI is determined more by the exigencies of cladoceran life histories than by the presence of fish. Nevertheless, absolute energy investment is depressed in the high-predation lakes and we suggest that clonal replacement mediated by size-selective predation by the fish can account for the differences between the lake types.

Les auteurs ont étudié certains aspects de la dynamique reproductive de *Holopedium gibberum* dans 25 lacs du parc Algonquin, en Ontario. À l'aide d'un indice basé sur la taille des *Daphnia*, ils ont réparti les lacs en deux groupes caractérisés par une prédation par les poissons élevée ou faible. De façon générale, le volume des oeufs s'est avéré un bon paramètre de prévision des réserves énergétiques des oeufs de stade 1 de *Holopedium*. Les oeufs de petite taille, des petites femelles typiques des lacs à forte prédation, contenaient relativement moins de lipides que les gros oeufs, ce qui porte à croire que la prédation par les poissons réduit indirectement la résistance au manque de nourriture des *Holopedium* nouvellement nés. De plus, dans les lacs contenant beaucoup de poissons, la taille moyenne des adultes, la taille minimale des femelles ovigères, les pontes et les volumes de graisse des *Holopedium* étaient inférieurs, comparativement aux lacs où la prédation par les poissons était faible. L'utilisation maternelle des lipides (UML) pour la production d'oeufs dans chacun des types de lac a été comparée. Les auteurs ont noté des écarts très apparents entre l'allure saisonnière de la valeur absolue de l'UML entre les deux groupes de lacs. Les écarts étaient surtout fonction des différences de tailles corporelles. La plus grande différence de UML absolue entre les deux types de lacs a été notée en mai, les femelles des lacs de faible prédation consacrant quatre fois plus de leurs réserves énergétiques à la reproduction, comparativement aux femelles des lacs de prédation élevée. La UML relative (réserves énergétiques par microgramme poids des femelles), qui ne compare que les individus dont les tailles se recouvrent, n'était pas fonction du type de lac. Une proportion constante de la masse des femelles ovigères est donc consacrée à la reproduction et l'on note une composante saisonnière fonction de l'abondance des algues communes, ceci dans bon nombre de lacs du Bouclier. Cela porte à croire que la UML relative est plus déterminée par les besoins du cycle vital des cladocères que par la présence de poissons. L'utilisation absolue d'énergie pour la reproduction est cependant réduite dans les lacs à prédation élevée et les auteurs formulent l'hypothèse qu'un remplacement clonal ayant pour cause la prédation sélective quant à la taille faite par les poissons pourrait expliquer les écarts entre les deux types de lacs.

Received February 2, 1988
Accepted July 28, 1988
(J9567)

Reçu le 2 février 1988
Accepté le 28 juillet 1988

Body size is reduced by the action of size-selective fish predators (Hrbáček 1962; Brooks and Dodson 1965; Wells 1970; Hutchinson 1971; Warshaw 1972; Vanni 1987); however, the direct and indirect effects of these size reductions on the amount and seasonal pattern of lipid allocated to the offspring of parthenogenic cladocerans remain largely unexamined. If fish predation affects maternal lipid investment in zooplankton through reduction in body size, there will likely be important indirect consequences for early survival ability, and hence community structure, since early life stages of zoo-

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plankton are the most susceptible to starvation and are often a "bottleneck" to population growth (Neill 1975; Threlkeld 1976).

The eggs of *Holopedium gibberum*, as with most planktonic cladocerans, are carried in a transparent brood chamber and progress through five developmental stages (Threlkeld 1979). In the first developmental stage the eggs have a single spherical lipid droplet. In freshwater Cladocera these lipids have been characterized as neutral lipids belonging to the triacylglycerol family (Goulden and Henry 1984).

This lipid reserve provides energy to the embryo throughout early development. The fat remaining once the animal is released from the brood chamber is available to sustain the neonate through early periods of food shortage. Neonates of larger species are born with disproportionally more lipid than neonates of small-bodied forms, and this extra energy reserve is correlated with enhanced early survival (Goulden et al. 1982). Goulden and Henry (1984) found that neonates of *Daphnia magna* had relatively more triacylglycerols and survived starvation longer (Tessier et al. 1983) than neonates of the smaller *D. galeata mendotae*.

In this paper we explore this critical aspect of early life, namely the energy investment into eggs of *Holopedium*, by measuring clutch size and lipid energy reserves allocated to eggs of *Holopedium* among lakes which vary in the intensity of fish predation.

Materials and Methods

We collected zooplankton from 22 lakes in May, an additional 3 lakes in June and August, and an additional 12 lakes in July of 1984 in Algonquin Provincial Park, Ontario (Table 1). The lakes, located on granitic or conglomerate and derived-metamorphic rock typical of the Canadian Shield, are oligotrophic and of circumneutral pH. Secchi disk transparency ranged from 1.3 to 6.6 m (mean 3.9 m), conductivity from 26 to 181 μmho/cm (mean 45 μmho/cm), and dissolved organic carbon from 2.2 to 8.2 mg/L (mean 4.34 mg/L) (Table 1).

Holopedium were caught using a large, tapered tow net (1 m^2 opening with 0.5-mm mesh) (Nero 1982) which was hauled from bottom to surface at the center of the deepest basin of each lake. *Holopedium* were immediately preserved in a cold, sucrose-saturated solution of 5% formaldehyde and stored in a styrofoam chest. *Daphnia* were sampled with a Wisconsin-style tow net with 30- and 45-cm diameter hoops and 2 m of 53-μm

TABLE 1. Selected physicochemical characteristics and indexing criteria (low predation followed by high predation, see text) of the study lakes from Algonquin Park, Ontario.

Lake	Lat.	Long.	Surface area (ha)	Secchi depth (m)	pH	DOC (mg/L)	Conductivity (μmho/cm)	*Daphnia* mean size (mm)	Mean % *Daphnia* >1.3 mm
Big Trout	45°46′N	78°37′W	1333.4	3.0	6.48	4.0	32	0.86	8.0
Billy	45°38′N	78°07′W	94.9	5.3	6.81	4.0	40	1.07	42.1
Brewer	45°35′N	78°19′W	38.8	4.0	6.37	4.6	68	0.86	12.4
Canisbay	45°34′N	78°35′W	161.4	4.3	6.33	3.8	34	1.01	15.3
Costello	45°35′N	78°19′W	7.5	3.5	6.29	4.9	54	0.86	14.9
Happy Isle	45°45′N	78°30′W	536.2	6.4	6.75	2.2	35	1.11	24.8
Heron	45°25′N	78°51′W	26.0	4.3	6.77	2.8	57	0.81	5.2
Kearney	45°34′N	78°26′W	34.6	3.6	6.28	5.2	43	0.94	17.6
Lamuir	45°50′N	78°35′W	740.6	4.2	6.72	3.6	38	1.11	34.0
Major[a]	45°37′N	78°06′W	26.3	6.6	6.89	—	54	1.12	36.6
Merchant	45°46′N	78°31′W	443.3	5.5	6.48	2.8	34	0.98	17.0
Mykiss	45°40′N	78°14′W	23.9	5.4	6.60	3.8	38	0.97	17.4
Provoking	45°34′N	78°30′W	110.4	4.1	6.05	3.9	29	0.83	7.3
Rock	45°31′N	78°24′W	499.3	4.0	6.36	—	36	1.04	30.2
Ryan	45°41′N	78°06′W	48.1	5.2	6.79	2.7	33	0.92	15.6
Two Rivers	45°35′N	78°29′W	790.0	3.0	6.37	4.4	38	1.02	27.6
Whitefish[a]	45°33′N	78°25′W	312.2	3.9	6.52	4.3	39	0.80	6.2
Big Crow	45°49′N	78°26′W	439.4	3.1	6.56	5.4	40	0.75	0.5
Booth	45°39′N	78°12′W	538.6	4.0	6.66	3.9	40	0.78	0.9
Bridle	45°40′N	78°09′W	22.0	3.5	6.72	4.9	51	0.58	0.0
Burnt Island	45°39′N	78°39′W	982.6	3.9	6.28	3.3	32	0.90	0.0
Catfish	45°57′N	78°33′W	652.0	3.0	6.43	4.2	33	0.59	0.0
Clarke[a]	45°32′N	78°16′W	26.0	2.5	6.35	—	53	0.70	1.1
Crotch[a]	45°39′N	78°06′W	268.8	4.0	6.45	—	32	0.68	0.0
Hogan	45°52′N	78°30′W	1624.4	3.3	6.78	4.3	40	0.60	0.0
Lavielle[a]	45°52′N	78°15′W	2549.6	3.9	6.90	—	57	0.79	0.0
Opeongo[a]	45°43′N	78°21′W	6018.4	6.0	6.39	—	36	0.74	1.1
Park	45°25′N	78°51′W	44.9	1.8	6.29	6.1	37	0.58	0.6
Poverty[a]	45°29′N	78°13′W	23.5	1.5	6.10	—	51	0.63	2.7
Proulx	45°46′N	78°24′W	383.5	3.0	6.71	5.1	41	0.62	0.0
Sasajewan	45°35′N	78°31′W	35.9	1.3	6.30	8.2	40	0.75	2.6
Smoke[a]	45°31′N	78°41′W	598.4	4.8	6.47	—	26	0.75	1.9
West Smith[a]	45°34′N	78°17′W	18.0	3.0	6.28	—	181	0.69	0.0
Wright	45°45′N	78°17′W	64.9	2.8	6.59	6.0	39	0.69	0.0

[a]Data collected this study, 1984. Remaining data collected by the Fisheries Acidification Study Program, 1982, the Ontario Ministry of Natural Resources, Research Section, Fisheries Branch, Maple, Ont. L0J 1E0.

mesh, hauled from bottom to surface in the deepest basin. All collections were made between 0800 and 1800.

From each lake, and on each sampling date, we systematically removed, whenever possible, 50 gravid *Holopedium* from a Petri dish etched with a square grid (0.5 cm). If, after removing 50 animals, there were still fewer than 10 of these carrying stage 1 eggs, we continued to examine females until we obtained, where possible, at least 10 females carrying stage 1 eggs. Carapace length of *Holopedium* was measured from the dorsal margin of the carapace immediately above the eye to the posterior tip of the carapace for animals in the contracted (preserved) position (Yan and Mackie 1987).

Minimum length of ovigerous females (OL_{min}) was defined as the mean length of females in the lower 10th length percentile (after Culver 1980) of the size distribution of ovigerous individuals (all egg stages) from a lake on a particular date. This is a conservative measure designed to eliminate the possibility of reliance on a single anomalous female to characterize OL_{min}. What we measured is not strictly the minimum length at first reproduction (L_{min}), but rather, the minimum ovigerous female size typical of a lake at a particular time (OL_{min}). In other words, we cannot determine from our data whether the observed clutch of a particular female is the first, or some later clutch; only the body size at the time the first clutch is released into the brood chamber can be correctly identified as the minimum size at first reproduction.

Clutch size was measured for females carrying stage 1 eggs only, because possible egg loss through abortion might reduce apparent clutch size for older clutches. Stage 1 egg and fat droplet diameters were measured using a compound microscope fitted with an ocular micrometer and converted to volumes assuming a spherical egg and fat droplet shape.

In the eggs of some cladocerans, e.g. *D. magna*, the lipid is dispersed in the yolk as several small droplets instead of a single droplet. We tested this for *Holopedium* by staining stage 1 eggs from females preserved in 5% sugared Formalin, with a triacylglycerol specific stain (Sudan IV red) according to the method of Bjorkman and Shapiro (1986). In all cases the only area in the egg to take up stain was the single large lipid droplet.

As an aid to future studies on maternal energy investment, we estimated sample sizes needed to obtain 95% confidence intervals (C.I.) around the mean egg volume, no wider than 1 and 2 nL, roughly representing a fine and a coarse level of precision. For most lakes (>90%), on a given month, we estimated that 50 and 20 females are sufficient to obtain 95% C.I.'s around the mean egg volume of 1- and 2-nL width, respectively.

Regression analysis, nested analysis of variance, and analysis of covariance were conducted using SAS Institute Inc. (1982) on a VAX 8200 computer. Sample sizes for 95% C.I.'s of a specified width and level of assurance (95%) were obtained as in Zar (1984). We used *t*-tests for differences between monthly means of percent fat in stage 1 eggs on data which were initially transformed using the arcsine transformation in order to satisfy the underlying assumption of linearity (Zar 1984).

Results and Discussion

Natural Variation in Egg Volumes and Maternal Lipid Investment

There was a significant linear relationship between fat droplet volume (stage 1 eggs) and total egg volume when all months and lakes were combined (Fig. 1). This regression (model I)

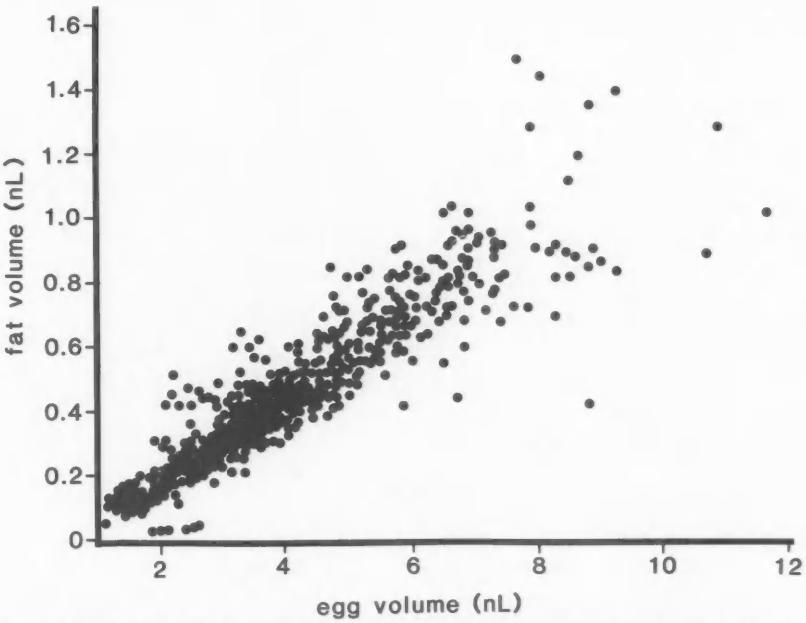

FIG. 1. Mean fat droplet volume versus mean egg volume of parthenogenic *Holopedium* carrying stage 1 eggs for all months and lakes combined. Each point is the mean fat volume and mean egg volume of a female. The regression equation is given by $Y = 0.126X - 0.0523$ ($n = 852$, $r^2 = 0.85$). The 95% C.I. for the slope is 0.126 ± 0.004.

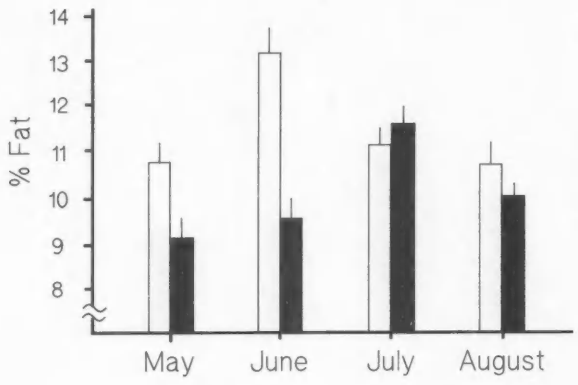

FIG. 2. Seasonal distribution of the relative proportion of fat (percent of total egg volume that is fat volume) in stage 1 eggs of *Holopedium* in low-predation (open bars) and high-predation (solid bars) lakes. Vertical lines above each bar are 95% C.I.'s

accounts for most of the variation in fat volume (r^2 = 85%), and the standard error of the regression is small (0.90), indicating that egg volume is a relatively good predictor of the fat volume in the eggs of this species.

Stage 1 eggs from low-predation lakes contained relatively more fat, on average, than eggs from high predation lakes in May, June, and August (*t*-test, $p < 0.001$) (Fig. 2). In July, however, the reverse is true ($p < 0.05$) (Fig. 2). We did not determine the exact magnitude of advantage that would accrue to an egg which possesses a relatively larger amount of fat; however, Goulden et al. (1987) have shown that eggs which have increased relative energy reserves (different *Daphnia* species) are able to resist starvation for longer periods. Also, neonates born from eggs with relatively more fat may benefit from increased buoyancy, since these neutral lipids float in water. Typically, absolute egg and fat volumes were larger in the low-predation lakes (Table 2).

In this paper we use the term "maternal lipid investment" (MLI) to describe the lipid energy reserves transferred from the mature female to the eggs at the time that they are deposited in the brood chamber. We defined MLI for a given female as the product of the average fat volume of the stage 1 eggs in her clutch and clutch size. Because *Holopedium* provides no parental care for its offspring beyond the egg stage, maternal investment in this species is represented largely by the energy content of its eggs (MLI) and the protection the brood chamber affords the developing embryo. We realize that by calculating means for MLI for each month across lakes that we are amalgamating energy investments from different individuals (perhaps different clones) which may differ in important attributes such as size, egg development times, or feeding success both within and between lakes. More work needs to be done under carefully controlled laboratory conditions, or in in situ enclosures, with respect to egg development times and survivorship of neonates of different clones of *Holopedium* in relation to MLI, both with and without fish. On the other hand, multilake surveys such as the one presented here may impart a realism unhampered by problems such as enclosure effects or artificial conditions inherent in laboratory culture studies.

In surveys of MLI in *Holopedium*, and possibly other Cladocera, most effort should be directed at sampling more lakes rather than collecting more females within a lake because although lake of origin and female within lake significantly

TABLE 2. Comparison of fat volumes, egg volumes, clutch size, and biomass-weighted MLI in low- and high-predation lakes. Sample size is the same for fat and egg volume and clutch. Significance was determined using the *t*-test (Zar 1984).

	May		June		July		August	
	Low	High	Low	High	Low	High	Low	High
n	114	69	106	110	138	128	116	71
Fat volume (nL)								
Mean	0.46	0.13	0.63	0.29	0.53	0.49	0.44	0.33
95% C.I.	0.05	0.01	0.05	0.03	0.03	0.03	0.03	0.03
Prob.	$p < 0.001$		$p < 0.001$		$p > 0.05$		$p < 0.001$	
Egg volume (nL)								
Mean	4.05	1.49	4.89	2.86	4.69	4.20	4.03	3.27
95% C.I.	0.31	0.06	0.35	0.22	0.24	0.28	0.22	0.24
Prob.	$p < 0.001$		$p < 0.001$		$p < 0.01$		$p < 0.001$	
Clutch size								
Mean	8.8	6.32	3.35	3.27	3.18	1.89	3.40	2.13
95% C.I.	1.19	0.72	0.64	0.52	0.37	0.20	0.35	0.18
Prob.	$p < 0.005$		$p > 0.05$		$p < 0.001$		$p < 0.001$	
n	53	34	35	72	79	48	78	47
Biomass-weighted MLI								
Mean	0.21	0.21	0.09	0.08	0.04	0.04	0.06	0.06
95% C.I.	0.03	0.02	0.02	0.01	0.01	0.01	0.01	0.01
Prob.	$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	

TABLE 3. Nested analysis of variance on egg volumes for each of 4 mo in 1984. *$p < 0.001$.

	May		June		July		August	
Source of variation	% total	F	% total	F	% total	F	% total	F
Among all females	97.5		98.7		97.2		96.2	
Lakes	79.5	42.2*	55.5	12.2*	82.4	52.4*	63.6	19.0*
Females in lake	18.0	55.5*	43.2	83.2*	14.8	8.7*	32.6	18.9*
Error	2.5		1.3		2.8		3.8	

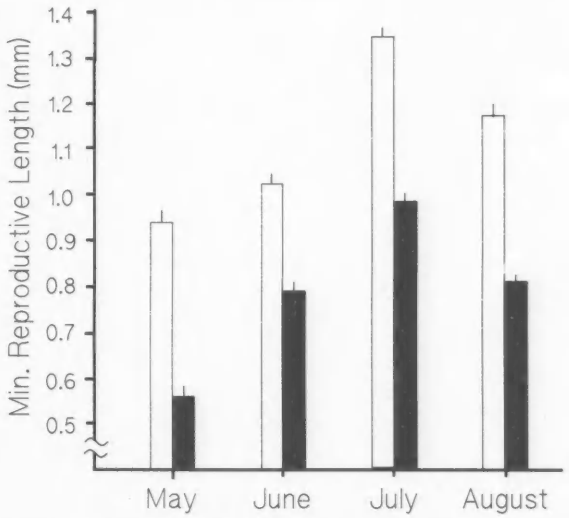

FIG. 3. Seasonal pattern of minimum reproductive length of *Holopedium* in low-predation (open bars) and high-predation (solid bars) lakes. Vertical lines above each bar are 95% C.I.'s. $p < 0.001$ for each comparison.

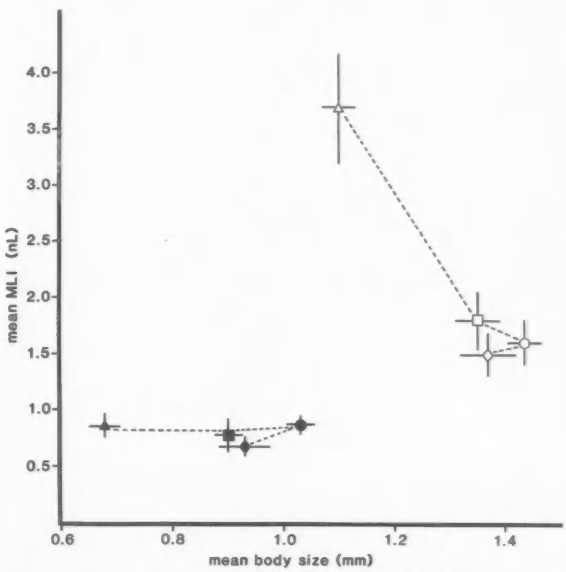

FIG. 4. Mean MLI and body length of ovigerous females in May (triangles), June (squares), July (circles), and August (diamonds). Closed and open symbols represent the high- and low-predation lakes, respectively. The broken lines connect the lakes in time. Solid vertical and horizontal bars are the 95% C.I.'s for mean MLI and mean body length, respectively. Sample sizes are 114, 106, 138, and 116 and 69, 110, 128, and 71 for the low- and high-predation lakes, respectively, from May to August.

affected egg volume, lake of origin accounted for greater than 50% of the overall variation (Table 3). Also, less than 4% of the total variation in egg volume is attributable to variation among eggs within a brood pouch (Table 3). Thus, the product of a single egg's fat drop volume and clutch size of its mother should suffice to characterize the extent of a female's MLI, regardless of clutch size.

Daphnia as an Index of Predation Risk

Adult and larval fish planktivores are usually size-selective visual feeders, and thus manifest their presence by a reduction in frequency of large-bodied forms and especially slower moving conspicuous cladocerans such as *Daphnia* (Hrbáček 1962; Brooks and Dodson 1965). Despite widespread support (Galbraith 1967; Wells 1970; Hutchinson 1971; Warshaw 1972; Werner and Hall 1974; Vanni 1987), these empirical observations are rarely used to predict effects on particular zooplankton species when standing stocks of adult planktivores are unknown. In addition, larval fish biomass is often ignored and correlations between adult fish biomass and the biomass of their larvae are not always viable because cohort strength of larval fish in any particular year may be influenced by catastrophic wind, current, and/or temperature episodes (Clady 1976).

Galbraith (1967 and 1975) and Mills and Schiavone (1982) used the relative frequency of large Cladocera (principally *Daphnia* species >1.3 mm) as an index of piscivore quality and as a guide to management policy. Similarly, we used the relative frequency of large-bodied *Daphnia* in each lake to classify that lake as having a *Daphnia* size distribution characteristic of a lake with a relatively high or low risk from fish (hereafter referred to as high and low predation) (Table 1). We designated lakes in which more than 5% of the *Daphnia* exceeded 1.3 mm as "low-risk" communities and those in which fewer than 5% of the *Daphnia* were greater than 1.3 mm as "high-risk" communities (Table 1). Our use of 1.3-mm *Daphnia* size is somewhat arbitrary; however, essentially similar results were obtained when 1.1- or 1.2-mm sizes were used as a cutoff. Also, yellow perch (*Perca flavescens*), lake trout (*Salvelinus namaycush*), and brook trout (*Salvelinus fontinalis*), which are common planktivores in the Algonquin Park region, have demonstrated preferences (by July for young-of-the-year fish) for mid-sized *Daphnia* of approximately 1.3-mm body length or greater (perch: Galbraith 1967; Wong and Ward 1972; Hanson and Wahl 1981; Mills et al. 1984; trout: Galbraith 1967; Northcote and Clarotto 1975; Kitchell and Kitchell 1980; Konkle and Sprules 1986). We used 5%, a conservative measure in parallel with statistical convention, because even in lakes with

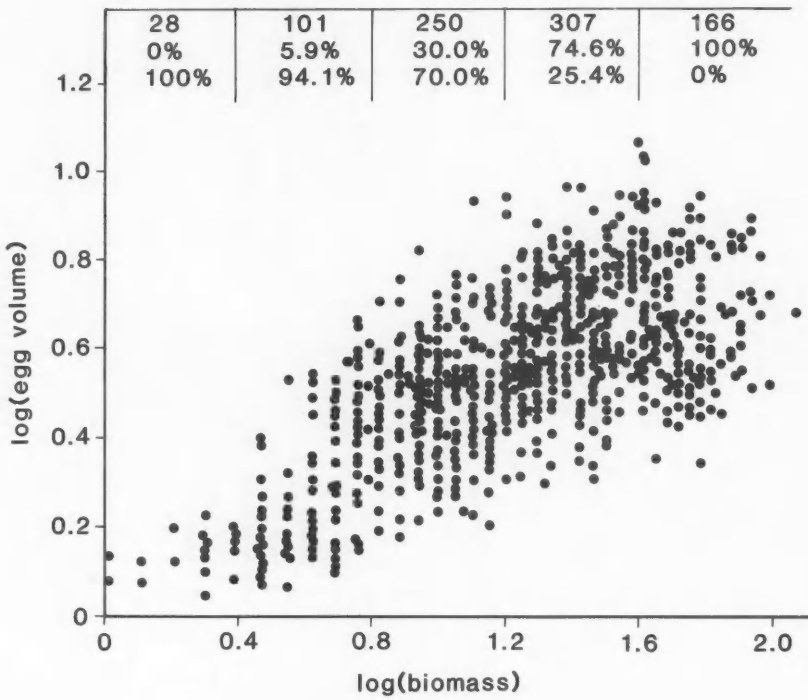

FIG. 5. Relationship between female size (gravid with stage 1 eggs) and egg size in *Holopedium* for all months pooled. The upper, middle, and lower numbers are the sample size and relative number of females from the low- and high-predation lakes, respectively, that fall within each equal logarithmic biomass interval. The model 1 regression ($n = 852$) is given by $Y = 0.3389\,X + 0.1326$, ± 0.024 (95% C.I. for coefficient). $r^2 = 0.47$.

high levels of planktivory there can be a small percentage of large *Daphnia* (Wells 1970; Evans and Jude 1986) that may have either escaped predation or recently immigrated. Although this index might be affected by the relative contribution of juveniles, for example if we had sampled immediately following a reproductive peak when large numbers of neonates could skew the size distribution, the fact the we reported a lake as high predation, only if less than 5% of the females were greater than 1.3 mm in any month (May to August), reduces the effect of this bias.

Predation Risk and Maternal Lipid Investment in *Holopedium*

Both Langford and Martin (1941) and Tessier (1986a) showed that *Holopedium* is vulnerable to predation by yellow perch, principally in July. Biomass of *Holopedium* was sharply reduced in the high-predation lakes in July, coincident with the increase in mouth gape (Arts and Evans 1987) and positive selection for *Holopedium* characteristic of the perch at this time (Arts and Sprules 1988). Perch occur in 34 of the 35 study lakes, are pelagic from June to early August, and have high fecundities (mean of 23 000 eggs/nest, range of 2000–90 000; Scott and Crossman 1973). Thus, it is likely that yellow perch have a significant impact on *Holopedium* in these lakes, although in other lake systems the presence of different fish predators such as roach (*Leuciscus rutilus*) and Eurasian perch (*Perca fluviatilis*) (Stenson 1973), whitefish (Coregonidae) (O'Brien 1975), and golden shiner (*Notemigonus crysoleucas*) (Tessier 1986b) also reduced mean size of *Holopedium*.

It has been proposed that fish predation can reduce size at first reproduction (L_{min}) of some zooplankton (Dodson 1974; Culver 1980; Vanni 1987). The OL_{min} of *Holopedium* is significantly reduced in the high- versus low-predation lakes in any pair of months (*t*-test, $p < 0.0001$) (Fig. 3). This observation lends support to the hypothesis that fish predation acts to selectively eliminate large-bodied clones resulting in populations with reduced OL_{min}'s. Culver (1980) proposed that temperature and not predation was the proximate cause for the seasonal reduction in L_{min}. However, the high- and low-predation lakes occur in the same geographic area and are represented by similar numbers and size ranges of lakes (Table 1), so that summer epilimnetic temperatures are similar for each group (M.T. Arts, unpubl. data).

Mean body lengths of *Holopedium* and mean MLI were both significantly different between high- and low-predation risk lakes (*t*-test, $p < 0.001$ between any pair of months) (Fig. 4).

Females in high-predation lakes were smaller and allocated less energy to the average clutch than did females from low-predation lakes (Fig. 4). The seasonal pattern of MLI differed markedly between the two lake types (Fig. 4). In high-predation lakes, mean MLI was depressed and remained relatively constant throughout the season at roughly 1.0 nL/female. In contrast, MLI was initially elevated in the low-predation lakes in May, but dropped sharply from 4.0 nL/female to between 1.4 and 1.8 nL/female in the June to August period (still significantly greater than the highest mean investment in the high-predation lakes). Thus, the reduction in MLI proved to be a combined effect of absolute fat volume and clutch size, since both were reduced (although not significantly for clutch size in June and fat volume in July) in the high-predation lakes (Table 3).

Although absolute MLI differed between the two lake types between any given month (Fig. 4) the relative amount of fat (nanolitres of fat per microgram wet weight of gravid female) did not differ when the data set was constrained to compare only females in a range of overlapping body lengths in a given month (Table 3). The seasonal pattern of relative per capita MLI followed the same trends between the two lake types. In both lake types the relative MLI was elevated at roughly 0.21 nL fat/μg tissue in May and then dropping abruptly to 0.09 and 0.04 nL fat/μg tissue in June and July, respectively. This was followed by a slight, but significant, increase in August to 0.06 nL fat/μg tissue. The similarity in these trends between the high- and low-predation lakes suggests that the basic dynamics of energy acquisition (relative MLI) are not substantially influenced by the presence of predators at a higher trophic level but rather may be a reflection of seasonal patterns in the underlying algal resource. Thus, the differences in the seasonal pattern of absolute MLI (Fig. 4) were largely the result of differences in body size.

Implications for Individuals

There are three important costs associated with being born to a smaller-bodied adult. First, the ability of zooplankton to allocate vital energy reserves to their offspring depends in part on the size range of ingestible particles and filtering rate which in turn depends on body size (Burns 1968 and 1969; Hall et al. 1976). Goulden et al. (1987), for example, found that females of large and small *Daphnia* species (interspecific) allocated fat to their eggs in proportion to their body size. In addition, respiration rates for small *Daphnia* embryos are relatively higher throughout development than for large *Daphnia* embryos, with the result that smaller neonates are born with relatively less fat and presumably reduced ability to withstand starvation (Goulden et al. 1987). We find, however, that large eggs of *Holopedium* (intraspecific), which are positively associated with large female size typical of the low-predation lakes (Fig. 5), typically have relatively more fat than small eggs (Fig. 2). Thus, in addition to the higher respiratory costs incurred by smaller developing embryos (Goulden et al. 1987), small eggs of *Holopedium* had relatively less fat than large eggs at the outset of development (Fig. 1 and 2). This suggests that in the high-predator lakes, *Holopedium* neonates would be less resistant to starvation. Starvation is likely to be increasingly important late in summer as the pulse of algal biomass generated by nutrients released during spring turnover decreases.

Second, invertebrate predators are constrained by the size of the feeding basket formed by their mouthparts to feed on smaller zooplankton (Kerfoot 1977; Wong 1981). *Holopedium* is susceptible to predation by the invertebrate predator *Chaoborus*, particularly by later instars (Fedorenko 1975). Lynch (1980), in defining optimal body size, noted that ecological fitness (E_x) would be greatest at that body size (x) where the product of survival probability (P_x) and feeding efficiency (F_x) for an individual is maximized. In the high-predation lakes, therefore, small size at birth implies increased risk to invertebrate predators, in particular *Chaoborus*, because time required to grow to optimal body size is likely increased. Although *Chaoborus americanus* shows a strong negative association with fish, other species such as *C. flavicans* and *C. punctipennis* which are commonly found in both lake types (M.T. Arts, unpubl. data) are more successful at avoiding predation by fish through behavioral avoidance mechanisms such as diel vertical migration (Von Ende 1979).

The small size (stage 1 females only) at the beginning of spring in the high-predation lakes (Fig. 4), before predation by perch is intense, suggests a carry-over effect from the previous season (smaller adults in the fall presumably producing smaller overwintering eggs which hatch into small-bodied progeny) and further reinforces the hypothesis of clonal replacement. Although we did not measure the sizes of resting eggs and the resulting sizes of neonates of these resting eggs relative to the sizes of their mothers, it is possible that individuals hatching from resting eggs deposited by the larger-bodied *Holopedium* of the low-predation lakes would be larger. Larger neonates would be more effective at utilizing the algal bloom initiated by spring turnover because they would reach the higher assimilation and ingestion rates associated with larger body size more quickly (for example see Lynch 1986). The suggestion that larger hatching size from resting eggs results in larger juveniles and adults with higher feeding rates is supported because mean body size and MLI of gravid females in May in the low-predation lakes are significantly higher than mean body size and MLI of gravid females from the high-predation lakes (Fig. 4). This occurred even though juveniles were first observed at about the same time in both lake types after ice-off when lakes of similar size were compared.

Implications for Populations

Havel (1987) showed the existence of different clones of *H. gibberum* in a study of 25 populations in Michigan and Wisconsin. We suggest that small-bodied *Holopedium* clones may replace large-bodied morphs due to size-selective fish predation. The fact that OL_{min} is reduced in the high- compared with the low-predation lakes suggests selection, over many generations, for smaller-sized females or clones. Lynch (1986) suggested that size at maturity has a strong regulatory influence on *Daphnia* life histories. All four *Daphnia* species in that study reached the species-specific L_{min} at first reproduction at nearly the same time and, having reached this size, allocated most of their net carbon intake (roughly 90%) into reproduction. However, for each species of *Daphnia*, ingestion and assimilation rates and absolute clutch size each showed a positive increase with body weight (Lynch 1986). Thus, it seems likely that individuals of the same species attaining L_{min} at relatively greater sizes should be able to allocate more energy (absolute) into growth and specifically reproduction (Lynch 1986). Although we did not calculate energy budgets for *Holopedium*, Lynch's (1986) findings raise the interesting possibility that the reduced minimum size of ovigerous *Holopedium*, through selection for

small-bodied clones in high- compared with low-predation lakes, may result in reduced energy allocation to reproduction at the individual level. The reduced MLI levels of individuals (Fig. 4) in the high- versus low-predation lakes support this.

Conclusions

Recently, there has been a proliferation of research on indirect effects of predators on their prey (Sih 1987). Chemically mediated morphological changes have been shown for invertebrate prey in the presence of their predators (Grant and Bayly 1981; Krueger and Dodson 1981; Stemberger and Gilbert 1984; Havel 1985). Complex behavioral interactions can exist between predators in two or more trophic levels and their common prey (Werner et al. 1983; Sih 1987). Predators, by their presence, may also disrupt the feeding of their prey (Folt and Goldman 1981). Here, we present another mechanism whereby predators can affect the growth and reproduction of their prey. *Holopedium* in high-predation lakes produced smaller eggs with relatively less fat, suggesting that starvation resistance of neonates is compromised in the presence of fish predators. Small body size (lower assimilation and ingestion rates) in the high-predation lakes was presumably carried over via resting eggs through the winter, resulting in a per capita MLI in the high-predation lakes that was 4 times lower than in low-predation lakes in May. In addition, the MLI pattern in the low-predation lakes remained about twice as high as that of the high-predation lakes for the remainder of the season. We hypothesize that in addition to direct consumption of prey, size-selective fish predation may result in subtle indirect effects on absolute maternal energy investment, and thus fitness, perhaps mediated through clonal replacement. Lake type had little effect on relative MLI (energy reserves per microgram female weight), suggesting that seasonal phytoplankton abundance rather than fish limits the relative capacity of *Holopedium* to allocate energy into reproduction.

Acknowledgments

We thank D. O. Evans, P. Johannes, B. Kuhkta, J. A. McClean, and J. Murdoch for logistic support. J. Hare and M. Pasitschniak-Arts helped with field work and enumeration of samples, respectively. N. C. Collins, D. A. Culver, M. Legner, C. W. Ramcharan, H. P. Riessen, D. A. Soluk, and N. D. Yan provided critical review on an early version of the manuscript. This study was funded by operating grants from the Natural Sciences and Engineering Research Council of Canada and an Ontario Ministry of Natural Resources Renewable Resource Grant to W. G. Sprules.

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Behaviour and Microhabitat of Young Atlantic Salmon (*Salmo salar*) during Winter

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Underwater observations at two sites along a small Nova Scotian river were carried out between December and April (water temperature range = 0.5–7.0°C) to describe the winter microhabitat of young Atlantic salmon (*Salmo salar*). Salmon (5–15 cm fork length) were consistently found hiding beneath rocks (mean diameter = 16.8–23.0 cm) in riffle-run habitats where mean water depths were 40.9–48.9 cm and mean water velocities were 38.7–45.7 cm·s^{-1}. Many of the salmon were found overwintering within redd excavations. "Home stones" were distributed closer to midstream than to river banks and where sediment compaction was minimal. Monthly collections of fish (ages 1 and 2) indicated that feeding continued over winter. The data suggest a nocturnal activity pattern and photonegative response by young salmon during winter.

On a fait des observations sous l'eau à deux endroits dans une petite rivière de la Nouvelle-Écosse entre les mois de décembre et d'avril (température de 0,5 à 7,0°C) en vue de connaître le micro-habitat du jeune saumon de l'Atlantique (*Salmo salar*) en hiver. On a toujours trouvé les saumons (longueur à la fourche : 5–15 cm) se cachant sous des pierres (diamètre moyen : 16,8–23,0 cm) sur des fonds ridés où la profondeur moyenne de l'eau était de 40,9–48,9 cm et la vitesse moyenne de l'écoulement, de 38,7–45,7 cm·s^{-1}. Bon nombre des saumons observés ont passé l'hiver dans des nids de frai. Les « pierres ancestrales » étaient plus près du milieu du lit que des berges et se trouvaient aux endroits où le tassement des sédiments était le plus faible. On a recueilli des poissons à tous les mois (classes de 1 et 2 ans) : on a constaté qu'ils continuaient de se nourrir durant l'hiver. D'après les données recueillies, le jeune saumon serait nocturne et aurait une réaction photonégative en hiver.

Received December 29, 1987
Accepted August 9, 1988
(J9535)

Reçu le 29 décembre 1987
Accepté le 9 août 1988

Descriptions of the behavioural changes adopted by young Atlantic salmon (*Salmo salar*) in rivers during winter are based on direct observations or inferences from information obtained during relatively brief periods. Gibson (1978) and Rimmer et al. (1983) described a microhabitat shift by salmon parr to positions beneath stream rubble as water temperatures decreased below 10°C in the autumn. Gardiner and Geddes (1980) saw few young salmon in a Scottish river during winter and assumed that they were hiding in the substrate. Smirnov et al. (1976) based their conclusions of winter activity of salmon parr in northern Soviet rivers on samples taken in March–April. There are no published accounts describing the behaviour and selected microhabitat of Atlantic salmon from the onset of hiding behaviour until its cessation in the spring. Despite such gaps in the data base, recommendations continue to be made on how to conserve salmon habitat in freshwater. Clearly, more information is needed for all periods of the year to increase the probability of success in salmon management. In the present study, I describe the winter behaviour and habitat of young Atlantic salmon based on a regular series of underwater observations and sample collections in South River, Nova Scotia.

Materials and Methods

The South River in Antigonish County, Nova Scotia (45°36′N, 61°54.8′W), is 27.5 km long, drains an area of approximately 200 km^2, and discharges into Northumberland Strait, Gulf of St. Lawrence. The average river gradient is 2.35 m·km^{-1}. During this study, mean monthly water temperatures and discharge ranged from 1.0°C (January) to 9.4°C (May) and 4.22 m^3·s^{-1} (January) to 12.70 m^3·s^{-1} (April), respectively (Water Resources Branch, Environment Canada, unpubl. data). Ice formation begins in early November and persists until late March, often covering entire stream sections, especially in the lower river. Occasional midwinter thaws can open the entire river system for short periods prior to refreezing. Anchor ice is often formed in open riffles. Major land-use activities along the river include farming, logging, and the quarrying of gravel and are attributed as being the cause of high sediment loading and discharge fluctuation in the system (Burton 1985).

Two study sites (A and B) were chosen (Table 1) which were generally representative of the river. A continuously recording thermograph at the Fraser's Mills provincial fish hatchery, adjacent to site A, monitored river temperatures. Water temperatures were also measured during each field trip. Descriptions of the habitat characteristics at each site (Table 1) were based on eight equidistant transects crossing the stream. Substrate descriptions were made by eye.

Fish species composition was the same at both sites. The most common species (summer) include Atlantic salmon, brook trout (*Salvelinus fontinalis*), gaspereau (*Alosa pseudoharengus*), white sucker (*Catostomus commersoni*), American eel (*Anguilla rostrata*), and three-spined stickleback (*Gasterosteus*

TABLE 1. Habitat characteristics at the two study sites on South River as measured during the winter of 1986–87. B = boulder; R = rubble; G = gravel; S = sand; Si = silt; M = macrophytes/moss.

Study site	Distance to river mouth (km)	Mean width (m)	Area (m^2)	Mean surface ice cover (%)[a]	Winter water temperature (°C)		Habitat type[b]	Substrate type (%)					
					Maximum	Minimum	Riffle : run : pool	B	R	G	S	Si	M
A	12.3	14.1	1353.6	28.0	3.0	0.5	2:4:1	7	35	40	9	2	7
B	8.5	15.4	693.0	45.6	3.0	0.1	2:6:1	4	24	62	7	3	0

[a]Percentage of wetted stream surface covered by ice as measured during winter field trips (December 11 to March 30).
[b]As defined by Rimmer et al. (1983).

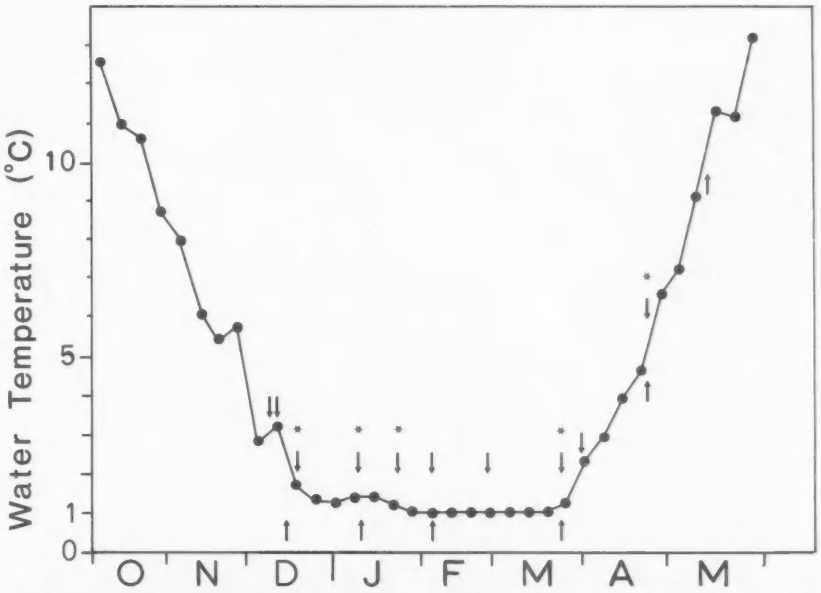

FIG. 1. Mean weekly water temperatures recorded in South River (at site A) during the winter of 1986–87. Arrows above the line indicate dates of snorkeling observations. Asterisks signify that both sites (A and B) were snorkeled on the same day. Arrows below the line indicate dates of sample collections.

aculeatus). In winter, only salmon and sticklebacks were commonly observed at the study sites. Anadromous Atlantic salmon commonly spawned within study site A, but not in site B.

Snorkeling observations were performed semimonthly at site A during winter (10 December 1986 to 30 March 1987) and once in April as mean weekly temperatures had increased above 5°C (Fig. 1). At site B, where greater ice cover and high water precluded as frequent a schedule, snorkeling was only accomplished semimonthly in January, once per month in December, March, and April, and not at all during February. Observations were made during the day between 0930 and 1500 using a drysuit, mask, and snorkel. The same zigzag transects (effective width = 2 m) were snorkeled each time with the diver moving in an upstream direction. The total transect lengths were 97 m in site A and 91 m in site B. A minimum underwater visibility of 1.5 m was required for a dive (Cunjak and Power 1986a). While moving along transects, the diver first searched for fish swimming or holding stations above the substrate before randomly overturning stones in search of fish. During this study, there was no evidence of having frightened a salmon to a position within the substrate during the diver's approach.

When a salmon was encountered hiding beneath a rock, species and size (±1 cm) were noted as per Cunjak and Power (1986a). Ages were assigned from size: young-of-the-year or age 1 = salmon ≤8 cm; all larger parr were assumed to be greater than age 1. Rock diameter, water depth, distance to shore, mean water velocity, and habitat type (i.e. riffle, run, or pool) were also recorded. Rock diameter (centimetres) was the distance along the longest axis of the "home stone" (Rimmer et al. 1984); water depth (centimetres) was measured from the water surface to the position of the fish beneath the home stone; distance to shore (metres) was the measured distance from home stone to nearest stream bank; and water velocity (centimetres per second) was measured in the water column at 0.60 of the total depth with an Ott "Pygmy" meter. During the first observations in December (site A only), a number of parr were found sheltering beneath stones in redds excavated the previous autumn. Subsequently, and until March 30 (last date of reliable identification of excavations), all parr sheltering in redds were recorded separately.

Juvenile salmon were sampled monthly from December to May (Fig. 1) by electrofishing at each site, immediately adjacent to snorkeling areas. Attempts were made to collect the first 10–12 salmon encountered. However, this was not always possible due to limited access to open water by ice. Each fish was

TABLE 2. Characteristics of the winter microhabitat of juvenile Atlantic salmon from two sites along South River, N.S. All measurements are mean values ± SE (range); n = number of fish observed.

Age	Study site	n	Fish size (cm)	Water depth (cm)	Home stone diameter (cm)	Water velocity ($cm \cdot s^{-1}$)	Distance from nearest bank[b] (m)
1	A	103	6.2 ± 0.1 (5 – 8)	44.5 ± 1.5 (31 – 65)	16.8 ± 0.5 (8 – 37)	45.7 ± 2.4 (5.3 – 110.0)	5.4 ± 0.2 (0.9 – 10.0)
	B	37	6.2 ± 0.1 (5 – 7)	48.9 ± 1.6 (22 – 83)	19.3 ± 1.2 (11 – 35)	40.8 ± 2.9 (9.0 – 101.6)	6.3 ± 0.3 (2.8 – 9.8)
≥2	A	7	11.6 ± 0.7 (10 – 15)	40.9 ± 3.1 (29–51)	23.0 ± 3.3 (15 – 41)	38.7 ± 5.0 (22.4 – 60.8)	4.5 ± 0.7 (1.6 – 6.4)
	B[a]	1	10.0	41.0	21.0	39.5	4.0

[a]No SE or range determined for variables because $n = 1$.

[b]Sample sizes slightly less for this variable at site A because measurements not taken on days of extensive ice cover (February); n = 90 and 6 for age 1 and ≥2, respectively.

measured (fork length (FL)), weighed, and condition factor was calculated; sex and state of maturity (immature, mature, or spent) were also determined. Stomachs were removed and stored in formalin for later diet analysis. A visual percentage estimate of stomach fullness was made after dissection. Prey items were identified at least to Order and the volume displacement (millilitres) measured.

Results

During 15 winter dives, the habitat use of 148 young salmon was described. Every salmon observed underwater in winter was hiding beneath a rock shelter. Water temperatures at the time of snorkeling ranged between 0.5°C (December–February) and 7°C (April 22). Young-of-the-year (age 1) salmon were much more numerous than parr (age ≥2), the latter accounting for only 7 and 2% of all salmon seen at sites A and B, respectively. Consequently, most of the data analysis deals with age 1 salmon.

Overwintering salmon ranged in size from 5 to 15 cm (Table 2). Intersite differences (age 1 only) were not significant ($|t| = 0.03, p > 0.95$). A correlation between fish size and home stone diameter was significant ($p = 0.002$) but accounted for only 6% of variability ($r = 0.251$). No significant correlations were found between the sizes of salmon observed and water depths ($r = 0.115, p = 0.164$) or water velocity ($r = -0.150, p = 0.069$).

Microhabitats chosen by salmon were generally in water 40–50 cm deep where the mean midcolumn water velocity was between 38 and 46 $cm \cdot s^{-1}$ (Table 2). Such conditions typify riffle-run habitats. Only two salmon were found in pool habitats. Home stone diameters averaged 17–23 cm (Table 2). Chosen microhabitats were similar between sites and between the two age classifications. Qualitatively, it was noted that most salmon located themselves under rocks which were "loose" on the bottom with many available accesses beneath. That is, home stones were not compacted along their edges by finer sediment particles. Although not measured, it is reasonable to assume that focal point velocities were close to zero beneath shelters, particularly because of the presence of fine sediments and detritus which would otherwise have been swept away.

At site A, many salmon were found hiding within redd excavations made that autumn. Of the 85 salmon found beneath rocks at site A (December to March), 33 (=39%) were located with redds. A total of 10 redds were identified within the transect area. Assuming a mean area of 1.90 m^2 per redd (Pratt 1968), redds represented only 10% of the total transect area of site A and yet accounted for almost 40% of the salmon observed.

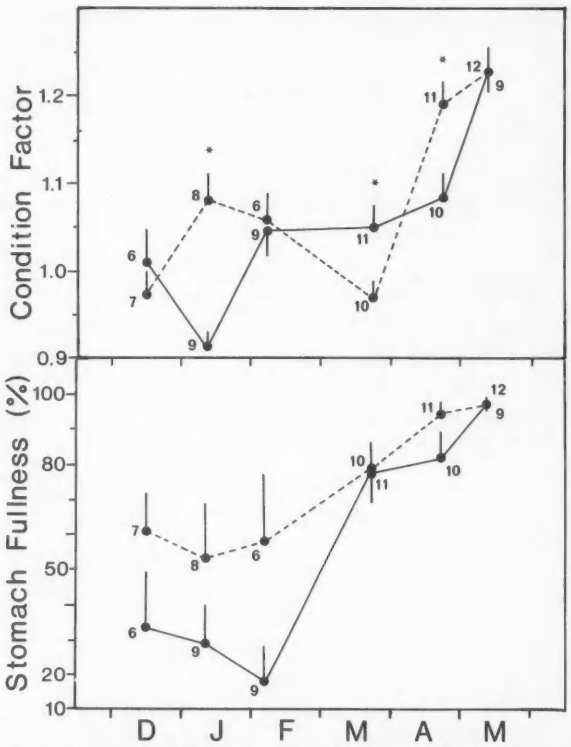

FIG. 2. Monthly changes in mean condition (upper panel) and mean stomach fullness (lower panel) for age 1 Atlantic salmon from site A (solid line) and site B (broken line). Vertical bars represent the standard errors; numbers indicate sample sizes. Asterisks represent significant ($p < 0.05$) intersite differences on specific sampling dates.

Most salmon that hid beneath rocks did so singly. However, four occurrences of two salmon and one occurrence of three salmon beneath common home stones were noted from both sites. The initial reaction, upon being uncovered, was to quickly swim away from the diver (usually downstream) and immediately seek shelter beneath another rock nearby.

The measured distances from home stone to stream bank permitted an analysis of instream distribution. A test of the observed frequencies for 0.5-m increments indicated that, instead of a homogenous distribution, home stones were concentrated towards midstream (site A: Kolmogorov–Smirnov, $D = 0.264$, $p < 0.001$; site B: $D = 0.379$, $p < 0.001$). Along dive transects at site A, within the first 3 m from either shore, <10% of the salmon were found compared with 51% for the 3 m measured from midstream toward either shore. At site B, the difference is similar; no salmon were found within 3 m of shore but 54% were near midstream. Mean population density estimates were based on the numbers of salmon uncovered during the winter observations. Mean density was 4.9 salmon·100 m^{-2} (range = 2.3–6.6) at site B which was significantly less ($|t| = 3.65$, $p < 0.01$) than the 9.5 salmon·100 m^{-2} (5.2–13.9) calculated at site A.

Feeding continued all winter (Fig. 2), with age 1 salmon (size range = 4.8–8.5 cm FL) collected from site B having higher mean indices of stomach fullness compared with site A, but differences were not significant ($p > 0.05$) on any date. At both sites, indices increased between February and May, suggesting that feeding rates also increased. Diversity of prey consumed in winter was low. Ephemeropteran nymphs (mainly Ephemerellidae) were most important accounting for at least 40% of the total diet (by volume). Trichoptera (mainly limnephilid and hydropsychid larvae) and Plecoptera (Perlodidae nymphs) were similarly important (5–14% of total) in the winter diets of young salmon. Condition factors (Fig. 2) of salmon (age 1) were variable but generally increased from winter to spring. The lowest condition factors were measured in January (site A) and on two dates (December and March) at site B. Significant intersite differences ($p < 0.05$) were measured in January, March, and April. Salmon parr (greater than age 1) were much scarcer than young-of-the-year and included precocious males (11) and immature parr (6 females and 2 males).

Discussion

Microhabitat measurements during winter were similar to those determined in late autumn by Rimmer et al. (1984) at the Little Sevogle River, N.B. Their study found that home stone size increased with fish age (0^+ to 3^+) and that most home stones were at least 20 cm in diameter in a narrow range of water depths (18–42 cm). These results led Rimmer et al. (1984) to conclude that substrate size followed by water depth were the primary criteria for determining habitat suitability. The present study confirms the importance of large substrates (i.e. boulders and rubble) for overwintering.

The importance of local environmental characteristics in influencing specific microhabitat selection is also evident. In South River, mean water velocities above home stones were as high as 110 cm·s^{-1} (age 1 salmon). The selection of microhabitats near high velocities is believed to be advantageous under prevailing conditions, specifically the high rate of sedimentation, from local land-use activities (Burton 1985), which tends to compact substrate particles. Because salmon seek refuge beneath stones, higher water velocities would reduce particle deposition, and thus compaction, by increasing sediment transport (Morisawa 1968). Cunjak and Power (1986b) suggested a similar reason to explain the use of high water velocity areas by *Rhinichthys atratulus* which also overwinters beneath stones. Because focal point water velocities beneath home stones are essentially zero (R. A. Cunjak, pers. obs.), energy expenditure would be minimal despite the fast curents nearby. This is especially advantageous at the low water temperatures of winter when a salmon's ability to hold position in flowing water is markedly reduced (Rimmer et al. 1985).

Areas in which redds are made provide many of the physical conditions required by young salmon for overwintering. Adult salmon, especially multi-sea-winter fish which predominate in South River (Chadwick et al. 1985), prefer digging redds where mean water depth is 38 cm, water velocities are between 25 and 90 cm·s^{-1} (Beland et al. 1982), and substrate composition is mainly rubble and gravel (Peterson 1978). These characteristics reflect the microhabitat conditions found around winter shelters. More importantly, digging activity tends to loosen stream particles (Field-Dodgson 1987) and increase permeability, especially in streams subject to high rates of sedimentation which can compact substrates (Peterson 1978). At site B, where no spawning occurs, the availability of suitable shelters may be limited because of compacted substrates, since suitably sized stones are present. Such site-specific influences should serve to impress upon managers the care necessary in adopting broad habitat guidelines for stream fishes.

The continued feeding and the fluctuations in general body condition between December and April indicate some degree of activity by juvenile salmon in winter. Other workers (Smirnov et al. 1976; Riddell and Leggett 1981) also presented evidence of winter feeding by salmon. The fact that no salmon was ever observed above the substrate during daylight hours indicates that emergence from shelters to feed occurs between dusk and dawn and suggests a photonegative response. In laboratory experiments, Rimmer (1980) found juvenile salmon to be strongly photonegative in autumn at water temperatures below 12°C in contrast with a strong photopositive response in summer (Gibson 1966).

The maintenance of limited activity in winter rather than entering a state of torpor (e.g. Green and Farwell 1971) should be adaptive because of the variable conditions (i.e. discharge fluctuations, ice movements, and alternate freezing and thawing) experienced in some streams (Reimers 1963). Modern land-use activities such as logging and agriculture tend to exaggerate the seasonal variability of such riverine conditions and, too, the adaptiveness of an active winter existence. Evidence of metabolic deficits and nutritional insufficiencies experienced by stream salmonids in winter (Gardiner and Geddes 1980; Cunjak and Power 1987) provides further support for the necessities of continued activity, particularly feeding, in winter.

Acknowledgements

The assistance of D. MacLean, manager of Fraser's Mills provincial fish hatchery, and his staff is sincerely appreciated. Critical reviews by E. M. P. Chadwick, R. G. Randall, R. J. Gibson, and two anonymous reviewers greatly improved an earlier draft of the manuscript. Financial assistance was provided by the Pelagics Section, Department of Fisheries and Oceans, Moncton, N.B., and a visiting postdoctoral fellowship.

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Experimental Induction of Liver Tumours in Rainbow Trout (*Salmo gairdneri*) by Contaminated Sediment from Hamilton Harbour, Ontario

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Sediments from Hamilton Harbour and an uncontaminated control site were collected and extracted for organic contaminants to investigate the putative association between chemical contaminants and high incidences of fish tumours in Hamilton Harbour. Sediment extract from Hamilton Harbour had high levels of polynuclear aromatic hydrocarbons and was mutagenic in the Ames bacterial mutagenicity assay. In two sets of experiments using a trout sac-fry microinjection route of exposure, Hamilton Harbour sediment extract induced hepatocellular carcinomas in rainbow trout (*Salmo gairdneri*).

Afin d'étudier l'association présumée entre les contaminants chimiques et les forts taux de tumeurs des poissons du port d'Hamilton, des sédiments du port d'Hamilton et d'une zone témoin non contaminée ont été recueillis et ont servis à la préparation d'extraits dont on a dosé les contaminants organiques. Les extraits de sédiments du port d'Hamilton présentaient de fortes teneurs en hydrocarbures polynucléaires aromatiques, et ils avaient des propriétés mutagènes, d'après l'essai de mutagénicité bactérienne d'Ames. Au cours de deux ensembles d'essais basés sur l'exposition par microinjection d'alevins de truite, l'extrait de sédiments du port d'Hamilton a causé l'apparition des carcinomes hépatocellulaires chez la truite arc-en-ciel (*Salmo gairdneri*).

Received December 10, 1987
Accepted July 26, 1988
(J9524)

Reçu le 10 décembre 1987
Accepté le 26 juillet 1988

The discovery of high incidences of tumours among feral fish populations inhabiting freshwater and estuarine ecosystems has stimulated concern over the possible association of these diseases with waterborne carcinogens. Neoplasms found in feral fish include gonadal tumours (Sonstegard 1977; Dickman and Steele 1986), oral and skin papillomas and dermal tumours (Sonstegard 1977; Black 1983; Baumann et al. 1987), and liver tumours (Malins et al. 1984; Smith et al. 1979; Murchelano and Wolke 1985; Baumann et al. 1987). There is evidence that oncogenic virus or single-cell parasites are involved in the etiology of some fish neoplasms (Sonstegard 1977; Sonstegard and Sonstegard 1978; Dawe et al. 1979), but chemical contaminants have been implicated in the etiology of tumours found in several fish populations (Couch and Harshbarger 1985).

The primary evidence supporting an association between chemical contaminants and fish tumours has been the presence of carcinogenic compounds in sediments located near fish populations with high tumour frequencies (Black et al. 1983; Malins et al. 1984) or the presence of high levels of contaminants in tumour-bearing fish (Smith et al. 1979; Baumann et al. 1987). However, Mix (1986) stated that much of this evidence is circumstantial and does not clearly show a cause and effect relationship between the presence of contaminants and the development of malignant fish tumours. In a more experimental approach to this problem, Black et al. (1985) reported that extracts from contaminated sediments collected in the Buffalo River produced benign skin papillomas when painted on a small number of brown bullheads (*Ictalurus nebulosus*) and produced preneoplastic liver lesions when administered to brown bullheads in the diet.

This study represents an experimental approach to examining the putative association between fish tumours and chemical contaminants in sediments of Hamilton Harbour, a highly industrialized region of western Lake Ontario in which high frequencies of neoplasms have been reported among populations of brown bullheads and white suckers (*Catostomus commersoni*) (Sonstegard 1977; Cairns et al. 1983; Smith et al. 1986). We report the levels of selected organochlorine and polynuclear aromatic compounds (PAH's) in extracts from sediment samples collected in Hamilton Harbour and at a control site. These sediment extracts were tested for in vitro mutagenic activity using the *Salmonella*/mammalian microsome mutagenicity assay (Ames test) and were tested for in vivo carcinogenic activity in fish using the trout embryo microinjection assay.

Methods

Study Areas

Hamilton Harbour (Burlington Harbour) is an isolated embayment located in western Lake Ontario (Fig. 1), with a

FIG. 1. Location of Hamilton Harbour in relation to western Lake Ontario, and the location of the control site at South Bay (inset).

total surface area of approximately 215 ha and a mean depth of 13 m. It is surrounded by the communities of Hamilton and Burlington, and around the harbour are located heavy and light manufacturing industries, with the steel industry being the largest. According to the Ontario Ministry of the Environment (1974), the harbour receives industrial effluents (27 m^3/s), treated municipal sewage (3.2 m^3/s), and discharges from Great Lakes shipping.

The control site is located in South Bay, which is located on the southeastern side of Manitoulin Island in the Georgian Bay area of the Great Lakes (Fig. 1, inset). There is no industry in the area, and the nearest municipality of South Baymouth is 10 km from the control site.

Sediment Extract Preparation and Analysis

Sediment samples were collected with an Ekman grab at a site in the eastern basin of Hamilton Harbour and from a control site at McLennan's Creek in South Bay. Approximately 2 kg of wet sediment was extracted from each site by combining extracts from subsamples of 250–500 g. A subsample was placed in a 1-L beaker containing 150 g of Na_2SO_4, and 500 mL of 1:1 acetone:hexane was added to the beaker. After the contents were polytron homogenized at 10 000 rpm for 10 min, the extract was allowed to separate and was dried by passing through an 8-cm column of Na_2SO_4. This extraction procedure was repeated twice more using 500- and 300-mL volumes of acetone–hexane. The solvent remaining in the sediment was recovered by centrifuging for 10 min at 2000 rpm. All extracts were rotary evaporated to approximately 20 mL and subsamples were pooled. The pooled sample was rotary evaporated to 4 mL and 100 mL of pesticide-grade acetone added. This procedure was repeated and the final extract volume adjusted with acetone to 12 mL (South Bay) or 24 mL (Hamilton Harbour). The extracts were stored at 4°C in 2-mL aliquots in sealed glass ampoules.

TABLE 1. Concentrations of organochlorine pesticides (OC's), polychlorinated biphenyls (PCB's), and polynuclear aromatic hydrocarbons (PAH's) in sediments collected from Hamilton Harbour and South Bay. ND = not detected.

Compound	Hamilton Harbour (ng/g)	South Bay (ng/g)
OC's/PCB's		
Hexachlorobenzene[a]	0.6	ND
Aldrin	ND	ND
Dieldrin	ND	ND
Endrin	ND	ND
Chlordane	ND	ND
Mirex[b]	ND	ND
DDT's[c]	ND	ND
Methoxychlor	ND	ND
PCB	20.9	2.9
PAH's		
Naphthalene	2 030	ND
Acenaphthylene	334	ND
Acenaphthene	398	ND
Fluorene	1 392	ND
Phenanthrene	11 408	1.83
Fluoranthene	5 060	0.76
Pyrene	4 260	0.81
Benzanthracene	2 360[d]	1.80
Benz[*b*]fluoranthene	3 488	ND
Benz[*k*]fluoranthene	685	ND
Benz[*a*]pyrene	4 780	ND
Indeno[1,2,3-*c*,*d*]pyrene	387	ND
Dibenz[*a*,*h*]anthracene	320	ND
Benz[*g*,*h*,*i*]perylene	1 613	ND

[a]Total of α and γ isomers.
[b]Total of Mirex and Photomirex.
[c]Total of DDT, DDD, and DDE isomers (*o*,*p*′; *p*,*p*′).
[d]Total of chrysene and benzanthracene.

Subsamples (100 μL) of sediment extracts were prepared for analysis of PCB's and organochlorine (OC) pesticides. Interfering compounds were removed from the subsamples by adsorption chromatography on a 2 × 1.5 cm inside diameter silica gel column (3% deactivated). The column was eluted with 62 mL of hexane (PCB fraction) and 50 mL of benzene (OC pesticide fraction), and the fractions made up to 5 mL in isooctane. Fractions were analyzed by high-resolution gas chromatography on a Varian 4700 GC using dual 30-m capillary columns (DB-5, DB-1) and electron capture detectors.

For analysis of PAH's, subsamples (100 μL) of sediment extracts were diluted to 1 mL with isooctane and analyzed by GC/HRMS-SIM using a Finnigan 4510 GC/MS and an Incos data system. Quantitation of selected PAH's was performed by the external standardization technique.

Sediment extracts were prepared for mutagenicity and carcinogenicity tests by taking 2-mL aliquots of extracts dissolved in acetone and adding 4 mL of dimethyl sulfoxide (DMSO). Acetone was evaporated off under nitrogen flow, and extracts were further diluted with DMSO to the desired test concentration.

Mutagenesis Assay

The *Salmonella*/mammalian microsome mutagenicity assay was conducted as described by Maron and Ames (1983) using strains TA98 and TA100. Rat liver microsome solution (*S*-9) from Aroclor 1254-induced rats was purchased from Inter-

TABLE 2. Mutagenicity of extracts from Hamilton Harbour and South Bay sediments tested with TA100 and TA98 *Salmonella* strains, with and without S-9 activation.

	Mean no. of revertants (n = 3)			
	TA100		TA98	
Treament (g)[a]	With S-9	Without S-9	With S-9	Without S-9
Hamilton Harbour				
2.5	202	203	61	64
1.2	211	209	52	60
0.6	194	163	49	47
0.3	188	114	39	45
Spontaneous	102	105	33	39
South Bay				
20.0	90	Toxic[b]	44	32
10.0	104	30	32	38
5.0	101	32	38	32
2.5	93	57	40	38
Spontaneous	97	97	38	39

[a]Dose of sediment extract calculated as equivalent wet weight of sediment applied to test plate (gram equivalents).

[b]Toxicity indicated by complete inhibition of bacterial growth.

Medico of Willowdale, Ontario. The number of revertant colonies was determined over a range of four doses for each extract, with and without *S*-9. There were three replicate test plates at each dose.

Trout Carcinogenesis Assay

Rainbow trout embryos (*Salmo gairdneri*) of the Kamloops strain were microinjected with sediment extracts in the sac-fry stage of development as described by Metcalfe et al. (1988). Briefly, sac-fry taken 3 d after hatch were anesthetized by immersion in CO_2-saturated water and placed on dry filter paper. Sac-fry were injected in the yolk with a 0.5-μL volume of extract in DMSO using a 25-μL Hamilton syringe and a 31-gauge syringe needle. Injected sac-fry were immediately placed in water at 12°C to recover and raised in raceways to "swim-up" (start of exogenous feeding after yolk absorption). Trout were then transferred to successively larger tanks as they were raised over a period of 1 yr to a weight of greater than 200 g. Fish from all treatments were kept at the same temperature (10–15°C) and at uniform tank densities to ensure comparable feeding and growth rates in all treatments. Fish were fed commercial trout diets ad libitum.

There were two carcinogenesis experiments, started in August 1985 and February 1986. Both experiments were conducted in the same manner, using the same sediment extracts, although extracts were tested for a dose–response in Experiment II. The toxicity of sediment extracts was determined in preliminary short-term tests. The highest dose chosen for carcinogenicity testing was one half of the concentration found to cause 50% mortality (above controls) between the time of injection and swim-up (12 d). In control treatments, trout were microinjected with DMSO, the solvent used to dilute sediment extracts.

At the end of 1 yr, trout were sacrificed by an overdose of anesthetic (MS-222). The trout were examined externally and internally for grossly visible neoplasms, and the liver was examined and preserved in 10% formalin or Bouin's fixative for histological examination. The livers, which had been dissected into four or five pieces, were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Livers in which lesions had been observed grossly were extensively sectioned for histological confirmation of visual observations. All other livers were surveyed by cutting one section from the proximal face of each paraffin block and examining it microscopically for evidence of neoplasia.

The total incidence of hepatic neoplasms was determined by summing the results of visual and histological surveys. Only hepatic carcinomas were included in the total incidence data. The histologic classification of hepatic neoplasms was based upon the criteria of Hendricks et al. (1984). Basophilic hepatocarcinomas were distinguished from preneoplastic basophilic cell foci (nodules) by increased mitotic activity in hepatocytes, compression of surrounding tissue, and increased hepatic cord width (greater than two cells wide). The frequencies of hepatic carcinomas in trout were compared statistically by means of chi-square contingency analysis with a continuity correction, as outlined by Armitage (1971).

Results

Chemical Analysis of Sediment Extracts

The total wet weight of sediment extracted for the Hamilton Harbour sample was 1.44 kg, and the total extracted for South Bay sediment was 2.16 kg. As summarized in Table 1, the concentrations of PCB's and OC pesticides in sediments were relatively low. Hexachlorobenzene and PCB's were detected in Hamilton Harbour sediment, but only PCB's were detected in the control sediment. Levels of PCB's were highest in Hamilton Harbour sediment (ppb level).

The levels of PAH's analyzed in Hamilton Harbour sediment were high, with a concentration of benz[*a*]pyrene at 4.78 ppm (Table 1). The chrysene/benzanthracene peaks for the Hamilton Harbour sample did not give a baseline separation, so the value reported is a total of the two PAH's. The concentrations of PAH's in South Bay sediment were very low (ppb range), and none of the higher molecular weight PAH compounds were detected.

Mutagenicity of Sediment Extracts

As summarized in Table 2, mutagenic activity of sediment extracts in the Ames test was relatively low. The test dose was calculated as the equivalent wet weight (gram equivalents) of sediment represented by the volume (0.1 mL) of extract applied to each plate. This facilitates comparison of mutagenicity data for the two sediments and allows for easy interpretation of results in terms of the analytical data.

In tests of Hamilton Harbour extract using the TA100 *Salmonella* strain, there was a doubling of revertants above spontaneous levels and a dose–response, both with and without the addition of S-9. This indicates significant mutagenic activity by the criteria of Johnson and Hopke (1980). With strain TA98, Hamilton Harbour extract elevated the numbers of revertants, but not to twice the spontaneous value. Doses of Hamilton Harbour extract greater than 2.5 g-equiv. were toxic to the test strains.

There was no indication of mutagenic activity in tests with South Bay sediment extract. In some tests, the extract was toxic to the test strains, as indicated by numbers of revertants below spontaneous values, or total inhibition of bacterial growth.

TABLE 3. Numbers of fish with hepatic lesions and total percent of fish with hepatocarcinomas in rainbow trout necropsied 12 mo after injection at the sac fry stage with sediment extracts from Hamilton Harbour and South Bay.

Treatment[a]	n	Visual survey[b]	Histologic survey[c]		Total carcinomas[d]	
			Foci[e]	Carcinomas	No.	%
Experiment I						
DMSO control	15	0	0	0	0	0
South Bay (0.05 g)	62	0	0	0	0	0
Hamilton Harbour (0.006 g)	123	8	1	3	11	8.9
Experiment II						
DMSO control	168	0	0	0	0	0
South Bay (0.05 g)	131	0	0	0	0	0
South Bay (0.025 g)	98	0	0	0	0	0
South Bay (0.012 g)	88	0	0	0	0	0
Hamilton Harbour (0.012 g)	148	7	6	5	12	8.1
Hamilton Harbour (0.006 g)	124	3	4	2	5	4.0
Hamilton Harbour (0.003 g)	65	2	2	0	2	3.1

[a]Dose of sediment extracts calculated as equivalent wet weight of sediment injected into embryos (gram equivalents).

[b]Numbers of fish with hepatic lesions observed grossly. All gross observations confirmed by histological examination as hepatocellular carcinomas.

[c]Numbers of additional fish with hepatic lesions detected by histological survey.

[d]Total fish with hepatocarcinomas detected by visual survey plus histological survey.

[e]Nonmalignant basophilic lesions not included in the total for hepatocarcinomas.

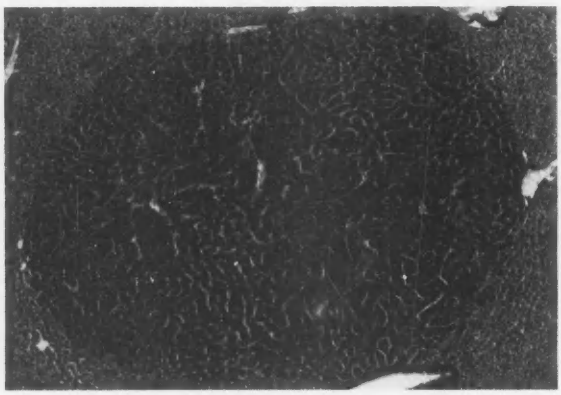

FIG. 2. Photomicrograph (magnification × 40) of a typical trabecular hepatocarcinoma induced in a rainbow trout 12 mo after injection of sac fry with Hamilton Harbour sediment extract (Experiment II, 0.012 g-equiv.).

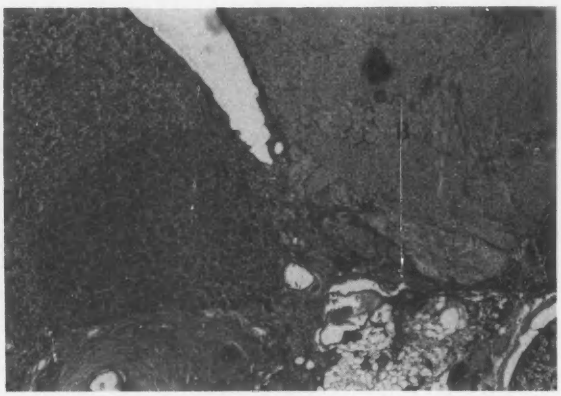

FIG. 3. Photomicrograph (magnification × 40) of an atypical hepatocarcinoma (A) and a sinusoid (B) observed in a rainbow trout 12 mo after injection of sac fry with Hamilton Harbour sediment extract (Experiment II, 0.003 g-equiv.).

Carcinogenicity of Sediment Extracts

The results of carcinogenicity Experiment I and Experiment II are summarized in Table 3. As with the Ames test data, doses are calculated as the equivalent wet weight of sediment (gram equivalents) represented by the volume of extract (0.5 μL) microinjected into each trout sac-fry. No grossly visible lesions, besides hepatic neoplasms, were noted externally or internally in any of the trout examined from Experiment I or Experiment II.

Data are presented on the number of hepatic neoplasms observed grossly, as well as the number of hepatic lesions (hepatocarcinomas, basophilic cell foci) observed in the histological survey. The total number of positives in each test represents the number of fish in which histologically confirmed hepatocarcinomas were observed. A previous study on the efficiency of this method of enumerating tumours (Metcalfe and Sonstegard 1985) indicated that visual plus histologic surveys detected over 90% of the trout with hepatocarcinomas, but the visual survey alone only detected 73%.

In Experiment I (Table 3), there were gross hepatic neoplasms seen in eight of the 123 trout which were necropsied 12 mo following microinjection with 0.006 g-equiv. of sediment extract from Hamilton Harbour. All of these neoplasms, which were relatively small (1–3 mm), whitish, nodular areas, were confirmed histologically as hepatocarcinomas. An additional three fish from this treatment had hepatocarcinomas which were not noted in the visual survey, and there was one fish with a

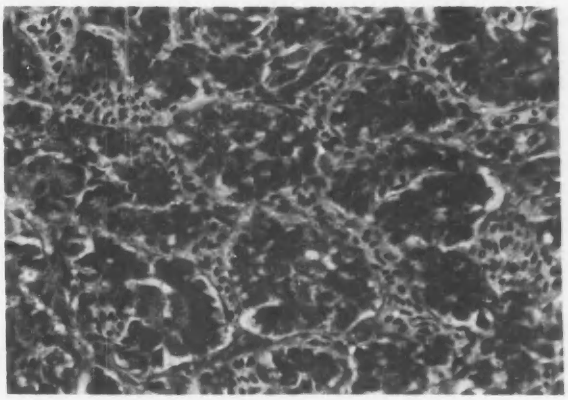

FIG. 4. Photomicrograph (magnification × 400) of the atypical hepatocarcinoma shown in Fig. 3.

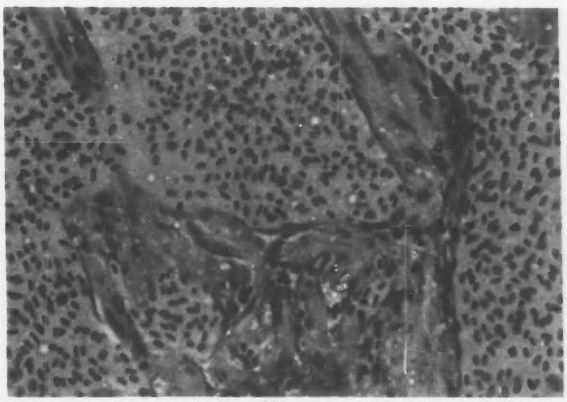

FIG. 5. Photomicrograph (magnification × 400) of the sinusoid shown in Fig. 3.

preneoplastic basophilic cell focus but no hepatocarcinoma. The total number of positives noted in the Hamilton Harbour treatment represented approximately 9% of the necropsied trout. No hepatic neoplasms were noted in trout which had been microinjected with South Bay sediment extract, or among control trout which had been microinjected with DMSO.

In Experiment II (Table 3), there were gross hepatic neoplasms observed in trout from all three treatments with Hamilton Harbour sediment extract, with the greatest number of gross neoplasms (7/148) observed among trout receiving the highest dose- (0.0125 g-equiv.). These lesions, which were confirmed histologically as hepatocarcinomas, were usually relatively small (1–3 mm), but in two cases were approximately 10 mm in diameter. Several additional fish from the Hamilton Harbour treatments had histologically visible hepatocarcinomas which were not observed in the initial visual survey (Table 3). When the results of the visual and histological surveys were combined, the percent positives noted among the Hamilton Harbour treatments in Experiment II showed a three-point dose–response. However, the difference between the percent carcinoma incidence at the two lowest doses was not statistically significant. Again, no hepatic neoplasms were noted in the treatments using South Bay sediment extracts or in the DMSO controls.

Histopathology

Histologically, the preneoplastic lesions observed in Hamilton Harbour treatments were basophilic cell foci, consisting of well-differentiated basophilic cells arranged in nodular areas of <1 to approximately 5 mm in diameter. The hepatocarcinomas noted in Hamilton Harbour treatments usually consisted of nodular areas of strongly basophilic hepatocytes arranged in a trabecular pattern (Fig. 2). There was compression of surrounding normal hepatocytes, as a result of rapid growth, and a few mitotic figures. With some of the larger trabeculate carcinomas, there were areas of hemorrhage in the center of the lesion, giving rise to erythrocytes free within the parenchyma.

There was one atypical hepatocellular carcinoma (Fig. 3), which consisted primarily of dense rosette formations of hepatocytes (Fig. 4). Adjacent to this lesion (Fig. 3) was an enlarged sinusoid containing blood cells and chords of stromal connective tissue (Fig. 5).

Discussion

In this study, we have shown that extracts from contaminated sediments are mutagenic in a bacterial assay and induce malignant hepatic tumours in a fish species, specifically rainbow trout. This is the first time that a complex environmental mixture has been shown to induce truly malignant neoplasms (hepatocarcinomas) in a fish carcinogenesis assay. The hepatocarcinomas induced in rainbow trout by chemical carcinogens are considered true malignancies by virtue of their potential for metastasis and their transplantability (Hendricks et al. 1981). However, the malignant nature of the lesion may not be apparent until late in the life of the fish, and the fish may die from other indirect effects of the tumour (e.g. hemorrhaging) well before the primary malignancy or metastatic lesions become life threatening.

The hepatocarcinomas and basophilic cell foci induced by Hamilton Harbour sediment extract resemble closely the lesions induced in rainbow trout by known carcinogens, such as aflatoxin B_1 and dimethylnitrosamine (Hendricks et al. 1984), benzo[*a*]pyrene (Hendricks et al. 1985; Black et al. 1985), and 7,12-dimethylbenzanthracene (Metcalfe nd Sonstegard 1984). Extracts from a control sediment did not induce hepatic neoplasms in these tests. A priori, it was considered possible that natural constituents of sediment could induce neoplasms, since Halver (1967) induced hepatomas in rainbow trout by long-term dietary exposure to tannic acids.

In addition to showing carcinogenic activity, Hamilton Harbour sediment extracts were mutagenic in the Ames test and contained high concentrations of PAH compounds. The levels of PAH's in this study are similar to those determined by Poulton (1987) for Hamilton Harbour and are similar to levels found in other highly polluted sediments (Black 1983; Malins et al. 1984; Baumann et al. 1987). Many of these PAH's, such as benz[*a*]pyrene, dibenz[*a*,*h*]anthracene, and benzanthracenes, are recognized mutagens/carcinogens. However, it cannot be inferred from these limited analytical data that PAH compounds were responsible for all of the mutagenic and carcinogenic activity in the extract. Ames test data showing that the Hamilton Harbour extract was mutagenic even without S-9 activation indicate that mutagenicity was at least partly due to the presence of direct-acting mutagens. PAH compounds are all indirect-acting mutagens/carcinogens, requiring addition of hepatic

enzymes (S-9) to Ames plates for metabolic activation (McCann et al. 1975).

In the trout microinjection assay, sac fry are capable of in vivo metabolic activation of indirect carcinogens, since the sac fry have a liver bud and a fully functioning oxidative enzyme system (Hendricks et al. 1981). Thus, carcinogenic activity could have been due to either indirect- or direct-acting compounds. If PAH compounds are responsible for the carcinogenic activity of Hamilton Harbour extract, it is possible to combine analytical and bioassay results to calculate dosimetry data. Assuming a benz[*a*]pyrene concentration of 4780 ng/g in the sediment extract (Table 1), a carcinogenic dose of 0.0125 g-equiv. of Hamilton Harbour sediment (Table 3) contains 60 ng or 0.06 μg of benz[*a*]pyrene per trout embryo. For comparison, Black et al. (1985) induced liver tumours in rainbow trout by embryo microinjection with 10 μg of benz[*a*]pyrene per embryo, and Metcalfe and Sonstegard (1984) induced liver tumours with microinjections of 250 ng per embryo of 7,12-dimethylbenz[*a*]anthracene and 13 ng per embryo of aflatoxin B_1.

There was a reduction in carcinogenic activity between the two experiments at the 0.006 g-equiv. dose (8.9 and 5.0% hepatocarcinomas, respectively). This may indicate some degradation of the active components of the mixture in the intervening 5 mo, even though the extracts were stored at 4°C in the dark. There was a positive response in the Ames test at 1.25 g-equiv. per test plate, a concentration two orders of magnitude higher than the dose per sac fry in the carcinogenicity assay. This illustrates the extreme sensitivity of the trout carcinogenicity assay, and also indicates the ease with which tumours could be initiated in situ among early life stages of feral fish.

These data showing the carcinogenic activity of sediment-bound contaminants to fish provide further evidence for an association between xenobiotics and the development of tumours in feral fish populations. In Hamilton Harbour, the incidence of lip and body papillomas among bottom-dwelling white suckers is elevated (Sonstegard 1977; Cairns 1983; Smith et al. 1986). There is evidence that C-type retroviruses are involved in the etiology of papillomas in suckers, although chemical contaminants may enhance the oncogenic expression of these viruses (Sonstegard 1977). Lip and body papillomas and epidermal carcinomas are also present in a high percentage of brown bullheads in Hamilton Harbour (Cairns 1983; Smith et al. 1986). Perhaps more indicative of chemically induced carcinogenesis in fish populations in Hamilton Harbour are the high incidences of hepatic tumours in white suckers and brown bullheads (Smith et al. 1986).

Although these data do not show that chemical contaminants are directly responsible for the development of neoplasms in fish from Hamilton Harbour, there is a clear indication of the potential for this etiology. This study also shows the flexibility and sensitivity of the trout sac fry carcinogenesis assay and indicates the potential for applying this technique to other problems in environmental toxicology.

Acknowledgments

This study was supported by operating and strategic grants from the Natural Sciences and Engineering Research Council of Canada to C.D.M. The authors wish to thank Michelle McWhirter, Gordon Balch, Bill Gibson, Jan Campfens, Ted Wolkowsky, and Nakos Kotsanis for technical assistance on the project. Histopathology was confirmed by Richard Moccia, Aquatic Pathology Services, Guelph, Ontario.

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Macroinvertebrate Communities and Environment in a Southern African Mountain Stream

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King, J. M., J. A. Day, P. R. Hurly, M-P. Henshall-Howard, and B. R. Davies. 1988. Macroinvertebrate communities and environment in a southern African mountain stream. Can. J. Fish. Aquat. Sci. 45: 2168–2181.

In a study of a second-order southern African stream, complementary classification and multidimensional scaling (MDS) techniques revealed longitudinal changes in macroinvertebrate community structure, but no temporal changes. Stepwise discriminant analysis and multiple linear regression were used to identify environmental variables correlated with the community changes but produced conflicting results depending on the information used, possibly because of strong correlations between some of the variables. The MDS plot of biotic samples illustrated that potassium levels correlated most strongly with community distribution. Because of the large number of variables now shown worldwide to correlate with faunal distributions, we suggest ways to choose the variables to suit the kind of study to be undertaken. Attempts to assign the invertebrates to functional feeding groups (FFGs) were unsatisfactory as the relevant categories are poorly defined and often inadequate for classifying the fauna. We suggest that until these categories are more clearly defined, and more uniformly applied, concepts relating to FFGs cannot be tested satisfactorily. The stream community in Langrivier is more similar to communities found in two other mediterranean ecosystems than to those in other southern African rivers, probably because of the greater predictability of flow in the former.

Dans une étude d'un cours d'eau secondaire de l'Afrique du Sud, des techniques de classification complémentaires et d'analyse multidimensionnelle (AM) ont mis en évidence des changements longitudinaux dans la structure des communautés de macro-invertébrés, mais aucun changement temporaire. Des techniques d'analyse par étape des paramètres et de régression linéaire multiple ont été utilisées pour identifier des variables environnementales corrélées avec les changements de la communauté, mais les résultats obtenus étaient contradictoires selon l'information utilisées, peut-être à cause de fortes corrélations entre certaines de variables. Le tracé MDS d'échantillons biotiques a montré que les teneurs en potassium présentaient de fortes corrélations avec la distribution communautaire. À cause du grand nombre de variables qui ne semblent pas, à l'échelle mondiale, présenter des corrélations avec les distributions fauniques, nous suggérons de choisir les variables en fonction du type d'étude à entreprendre. Les tentatives visant à attribuer des groupes nutritionnels fonctionnels (GNF) aux invertébrés n'ont pas donné des résultats satisfaisants, étant donné que les catégories pertinentes sont mal définies et souvent inadéquates pour classifier la faune. Nous croyons que jusqu'à ce que ces catégories soient plus clairement définies et plus uniformément appliquées, les hypothèses en rapport avec les GNF ne peuvent être vérifiées de façon satisfaisante. La communauté du cours d'eau de Langrivier ressemble plus aux communautés observées dans deux écosystèmes méditerranéens que celles d'autres rivières de l'Afrique du Sud, probablement à cause de la plus grande possibilité de prévision de l'écoulement.

Received January 15, 1988
Accepted July 26, 1988
(J9543)

Reçu le 15 janvier 1988
Accepté le 26 juillet 1988

In the last decade, numerical techniques have been used to an increasing extent to analyse large sets of biological data. In the field of lotic ecology, a variety of techniques has been used to identify distribution patterns of, and relationships between, biotic communities and to relate these to environmental data. Relevant studies have been reported from Australia (Lake et al. 1985b; Marchant et al. 1985; Bunn et al. 1986), New Zealand (Pridmore and Roper 1985), Great Britain (Townsend et al. 1983; Furse et al. 1984; Wright et al. 1984), North America (Roback et al. 1969: Kaesler and Cairns 1974; Gore 1980; Bruns et al. 1982; Cushing et al. 1983), and South Africa (King 1981).

In addition, considerable attention has been focussed on the functional organisation of stream invertebrate communities, and particularly on functional feeding groups (e.g. Cummins 1973; Cummins and Klug 1979; Anderson and Sedell 1979; Hawkins and Sedell 1981; Otto and Svensson 1983; Townsend et al. 1983; Lamberti and Moore 1984; Marchant et al. 1985; Bunn 1986). Riverine invertebrates are generally assumed to feed in a number of ways: some are predators, but most either graze epilithon or epiphyton, collect small organic particles from the substratum or water column, or shred larger pieces of dead organic matter such as leaves. It has been suggested that it should be possible to assign each invertebrate species to a functional feeding group (FFG) — basically predator, grazer, collector (gatherer or filterer), or shredder — and that the proportions of these groups should vary along a river in a way that reflects instream environmental changes, and thus changes in

the nature of organic energy (Vannote et al. 1980). Criticism of the FFG concept has come mainly from Southern Hemisphere lotic ecologists (Winterbourn et al. 1981; Lake et al. 1985a), possibly because of difficulties in assigning southern forms to FFGs originally derived from the northern fauna.

Both numerical and functional approaches to invertebrate community structure and distribution have the potential for allowing considerable insight into the ways in which stream ecosystems function and into their reactions to disturbance. We thus took a similar line when investigating and describing the invertebrate fauna of our study stream, Langrivier, choosing a variety of relevant techniques described in the literature.

Langrivier has been the subject of the first detailed study of lotic ecosystem functioning in an undisturbed catchment in South Africa (King et al. 1987a, 1987b). Such a study was initiated as part of the Fynbos Biome Project, which was designed to investigate the structure and functioning of a variety of ecosystems within this small but unique biome of sclerophyllous shrublands and heathlands (locally known as fynbos) at the southern tip of Africa (Day et al. 1979). The fynbos biome is one of five mediterranean ecosystems worldwide (Fig. 1), all of which are characterised by similar climates and vegetations and by their coastal positions at similar latitudes, usually at the southwestern limits of continents.

In this paper we describe the aquatic macroinvertebrate communities of Langrivier, indicate the relationships of these to environmental conditions, and initiate investigations of FFGs. Our aims were to establish if any spatial or temporal changes in community composition occurred in Langrivier, to relate any such changes to changing environmental conditions, and to determine the proportions of different FFGs for comparison with second-order streams elsewhere. We also wished to assess the similarity of Langrivier to streams in other mediterranean ecosystems.

Study Area

Langrivier is a second-order perennial stream within the fynbos biome (Fig. 1) and has been described by King et al. (1987a). It is 3 km long, 3–5 m wide, with mean discharges ranging from 0.03 $m^3 \cdot s^{-1}$ (summer) to 0.22 $m^3 \cdot s^{-1}$ (winter), and violent winter spates. Except during spates, recorded current speeds ranged from 0.19 to 1.56 $m \cdot s^{-1}$ and water depths at set points along the river from 12 to 50 cm. The streambed has a mean gradient of 17% and consists mainly of bedrock, boulders, and large stones. Finer materials are rare, with <10% of the streambed comprising sand and gravel. Water quality is very high, the concentrations of most ions being low and sometimes near the limit of detectability (unpublished data). Temperatures range from 8.0 to 20.0°C and pH values from 4.3 to 6.8, low pH values being characteristic of streams in the area.

Indigenous evergreen trees form a complete canopy over the stream, and the fall of their leaves through late spring and early summer forms the major source of instream organic material (King et al. 1987a). Between 1983 and 1985, levels of benthic organic matter (BOM) on, and up to 100 mm depth into, the streambed ranged from 8 g dry mass $\cdot m^{-2}$ in winter to 224 g dry mass $\cdot m^{-2}$ in summer, with 60% occurring as scattered coarse and fine BOM (>80 μm), 19% as ultrafine BOM (0.6–80 μm), and 21% as compact leaf-packs (King et al. 1987b). The only green plants visible in the stream are small patches of mosses. Leaf fall and high temperatures coincide, in contrast with the more commonly reported situation in the Northern Hemisphere, where autumnal leaf fall occurs at a time of decreasing temperatures.

Sampling stations, each encompassing a 50-m length of stream, were established at the only three accessible points along the stream. L1, at an altitude of 671 m, is situated below vertical cliffs in a ravine and close to the source of the stream. L2, at an altitude of 366 m and 2 km downstream of L1, is

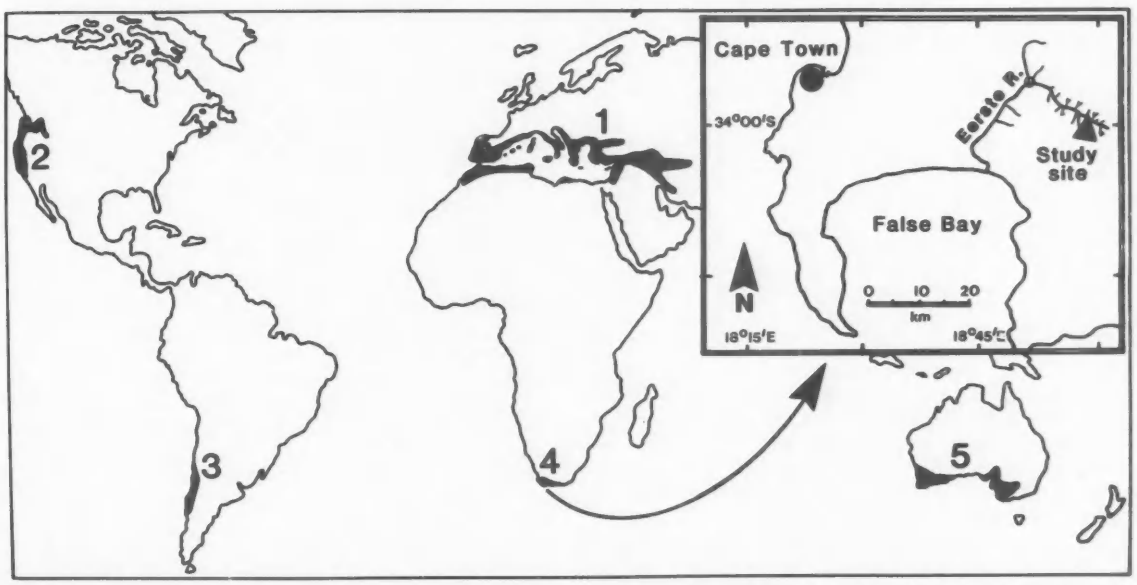

FIG. 1. Location of the five mediterranean ecosystems (Mollweider's interrupted homalographic projection) and of the study site in South Africa (inset). 1, Mediterranean Basin (vegetation type: macchia); 2, California (chaparral); 3, Chile (matorral); 4, southwestern Cape, South Africa (fynbos); 5, southwestern and southern Australia (heathlands).

located just upstream of a gauging weir and was used both for comparative monthly sampling and for all additional investigatory work. L3, at 335 m altitude and 0.5 km downstream of L2, is near the confluence of Langrivier with the Eerste River, the main river draining the Jonkershoek Valley.

Methods

Monthly visits were made to all three sites between April 1983 and May 1984 and then to L2 only for a further 18 mo. Measurements of many environmental variables were made and are reported elsewhere (leaf-packs: King et al. 1987b; allochthonous detritus: King et al. 1987a; papers on water chemistry, leaf decomposition, invertebrate life cycles, storm events, and drift, in preparation). Variables recorded in those papers and used here for analysis of environmental data were depth, current speed, BOM, temperature, pH, dissolved oxygen, total dissolved solids (TDS), conductivity, colour, hydrogen ion concentration, alkalinity, polyphenol concentration, and the ionic concentrations of sodium, potassium, calcium, magnesium, chloride, sulphate, silicate, nitrite, nitrate, ammonia, and soluble reactive phosphate. Benthic invertebrates were collected monthly using a square-sided sampler that covered 0.1 m^2 of streambed and incorporated a downstream net and collecting bottle, along the lines of that described by King (1981). The upstream side of the sampler was covered with netting of mesh size 950 μm, while the remaining sides and the collecting net were made of 80-μm-mesh netting.

Each month at each station, three faunal samples were taken from permanently inundated areas; as the stream appears similar throughout with no obvious pools or backwaters, these samples were judged to provide an adequate indication of faunal numbers and composition. Nevertheless, in October 1984, 16 separate samples were taken randomly throughout a single reach at L2, together with records of BOM, water depth, current speed, and habitat description, in order to determine if variations in microhabitat resulted in important variations in community structure. October was chosen for the intensive sampling because at that time current speeds start to decrease, the number of overwintering invertebrates on the streambed surface increases, but emergences have not yet begun. Samples were preserved in 70% alcohol and later the animals were sorted using a dissecting microscope. Identifications were taken to the lowest possible taxon, and biomass figures were obtained from average dry weights of medium-sized individuals in each case.

Invertebrates were categorised into the five FFGs in two ways in order to establish the proportions of the different groups along Langrivier. The fauna was assessed using the best local knowledge (e.g. Scholtz and Holm 1985) and the results of an investigation of foregut contents and mouthpart morphologies of those taxa contributing >3% of faunal numbers. In total, these taxa represented 90% of the number of macroinvertebrates in the stream. For each relevant taxon, 20 individuals were chosen from different months and sampling sites, their foreguts were mounted on microscope slides, and the contents analysed by a plant taxonomist. Mouthparts of three individuals of each taxon were similarly examined. In a second assessment, to investigate the validity of extrapolating from a North American data base, the animals were matched with "comparable North American species" (Marchant et al. 1985) using the guidelines to genera given by Merritt and Cummins (1984). Animals in families or genera with more than one given FFG were split between the relevant groups as described by Hawkins and Sedell (1981) and Bunn (1986).

Data were analysed numerically to detect changes in community composition along the stream and to relate these to changes in environmental conditions. Root–root transformed data of taxon abundance and complementary classification and multidimensional scaling (MDS) ordination techniques (Field et al. 1982) were used to cluster samples with similar biotic compositions. Indicator species characteristic of each group were then identified using information statistic tests (Field 1969). These tests were regarded by Field et al. (1982) as a useful way of reexamining the data, although they lack statistical rigour, the 5 or 1% probabilities of a species differing in occurrence between groups being best regarded as "arbitrary cutoff levels."

The relationships between the recognized biotic groups and a variety of environmental variables were investigated in two ways. Stepwise discriminant analysis, multiple linear regression, and analysis of variance (BMDP package, Dixon 1975) were used to investigate the degree of correlation between environmental variables, and between previously identified biotic groups and stated sets of environmental conditions. In stepwise discriminant analysis, a linear classification function of the environmental variables is constructed, which predicts the biotic group that should be present under a stated set of environmental conditions. The function constructed is that whose prediction best matches the observed biotic groups. When there are only two groups, as happened in this study, the multiple regression programme may be used as the problem reduces to one of multiple linear regression, with group number as the dependent variable. This programme also provides the correlation matrix for the environmental variables. Analysis of variance tests the equality of means of the environmental variables for the biotic groups. Other packages, such as SPSS (Hull and Nie 1981), were unavailable to us.

In a second investigation, using the procedure outlined by Field et al. (1982), the relationships between the biota and the environmental variables were displayed graphically by superimposing data for one environmental variable at a time on the MDS plot of biotic groups. In this way, the degree of correlation between a biotic group and a particular variable was visually displayed and environmental gradients that correlate with faunal distributions could be identified.

Results and Discussion

Numbers and Biomass

Animal numbers on and within the top 100 mm of the streambed ranged from 300·m^{-2} in the austral winter to more than 13 000·m^{-2} in spring, with the highest numbers usually occurring in November (late spring) (Fig. 2). Analysis of life cycle data will be reported upon elsewhere and here we merely comment on spatial and temporal changes in numbers. All three stations supported similar animal densities and showed similar peaks in numbers both in spring and in late summer, with the fluctuations in numbers occurring 1–2 mo later at L1 than at the two lower stations. The low summer peak in faunal numbers at L2 in the second summer (1984–85) additionally reflected a pattern that has shown up in several other sets of our data. In particular the volume of leaf fall was lowest in the same summer (King et al. 1987a), while rainfall in the preceding winter was lower in 1984 than in 1983 or 1985 (Forestry Branch, Department of Environmental Affairs).

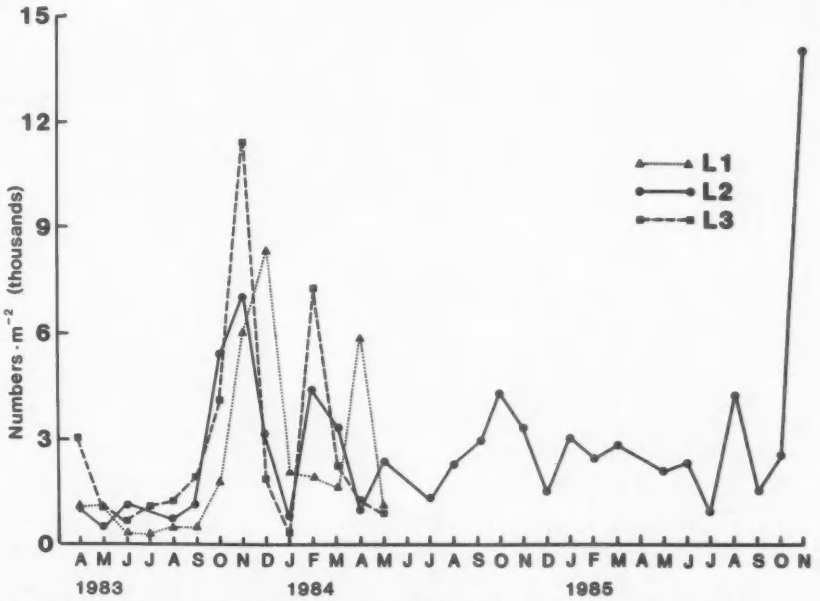

FIG. 2. Numbers of macroinvertebrates collected at three sites (L1, L2, L3) on Langrivier. Collections were made at all three sites for 14 mo and at L2 for a further 18 mo.

TABLE 1. Average (and range) percent composition by numbers of the invertebrate fauna at each site on Langrivier over 14 mo. Taxa comprising <1% of mean numbers over the study period at all three stations are not tabulated, but include the ephemeropteran *Lithogloea harrisoni*, the megalopterans *Taeniochauliodes* sp. and *Chloroniella peringueyi*, the trichopterans *Petrothrincus circularis*, *Chimarra* spp., *Parecnomina* ? *resina*, *Hydrosalpinx sericea*, *Athripsodes bergensis* group, *Dyschimus thrymmifer* and two other species, a tipulid and a ceratopogonid, at least eight unidentified coleopterans, and the odonates *Aeschna* sp. and a zygopteran. An asterisk denotes one record only.

Species	L1	L2	L3
Ephemeroptera			
Castanophlebia calida	3 (2.9–13.1)	10 (0.9–32.4)	9 (1.9–23.9)
Other Leptophlebiidae	6 (0.3–14.8)	2 (0–9.7)	2 (0–6.1)
Lestagella penicillata	1 (0–8.9)	2 (0–6.7)	2 (0–6.8)
Acentrella capensis	3 (0–7.2)	6 (0–17.0)	10 (0.5–18.5)
Baetis ? *harrisoni*	<1 (0–1.2)	3 (0–8.7)	6 (0–11.1)
Plecoptera			
Aphanicerca spp.	19 (5.2–44.9)	20 (8.4–42.5)	18 (6.0–25.9)
Trichoptera			
Petrothrincus triangularis	3 (0–21.9)	<1*	<1*
Cheumatopsyche spp.	<1 (0–0.9)	<1 (0–3.8)	1 (0.5–3.6)
Agapetus sp.	<1 (0–7.2)	1 (0–15.9)	6 (0.1–20.7)
Diptera			
Simuliidae	12 (0–19.3)	21 (2.4–55.3)	13 (1.5–40.2)
Athericidae	2 (1.3–6.4)	<1 (0–0.3)	<1 (0–0.3)
Blephariceridae	<1 (0–9.9)	3 (0–12.1)	3 (0–16.8)
Chironomidae	13 (0–49.7)	21 (0–74.9)	14 (0–46.0)
Coleoptera			
Hydraenid adult B	12 (0–26.7)	<1*	<1 (0–4.2)
Dryopid adult A	2 (0.1–14.0)	<1*	<1 (0–0.5)
Elmid larva B	1 (0–11.3)	<1 (0–2.1)	<1 (0–7.2)
Elmid larva C	7 (0–25.8)	<1 (0–1.6)	2 (0–5.5)
Helodid larva A	<1 (0–6.3)	<1 (0–11.8)	1 (0–10.0)
Helodid larva D	8 (2.2–14.8)	6 (0–15.8)	5 (0.2–13.6)

Numbers could not be compared directly with those reported for other studies because there is little conformity in the mesh size of collecting nets. It seems probable, however, that densities in Langrivier were similar to those in the headwaters of the main river draining the valley (the Eerste River, King 1983), if one takes into account the coarser netting used in that study and the fact that densities were notably higher in Langrivier than in the Eerste River only during the main time of juvenile recruitment (November), when the finer net would have collected hatchlings missed by the coarser net. Densities in the Eerste River were in turn similar to those reported by Bunn et al. (1986), who used similar meshed netting when sampling streams of the mediterranean ecosystem in Western Australia, but lower than in many streams elsewhere (for comparisons, see King 1983).

Biomass values for total macroinvertebrates ranged from winter lows of about 0.1 g dry mass·m^{-2} to early summer highs of 2.5 g dry mass·m^{-2}, with an annual mean of 0.6 g·m^{-2}. It should be emphasized, however, that values for both numbers and biomass reflect the animals that could be reached, and thus sampled, in this extremely rugged streambed, rather than absolute values. Data on density, biomass, and life cycles (unpublished) of Langrivier invertebrates support the finding of Harrison and Elsworth (1958) and King (1983) that acid, oligotrophic streams of the southwestern Cape are low in standing stock and productivity.

Species Composition

As habitats such as marginal vegetation, algal mats, and quiet backwaters were nonexistent, animals of the stony bed adequately represented the invertebrate fauna of the stream as a whole. Throughout the stream, insects accounted for >98% of the benthic invertebrates by numbers (Table 1), with the Diptera most abundant (34% of all individuals collected), followed by the Ephemeroptera (21%), Plecoptera (19%), Coleoptera (14%), Trichoptera (5%), Megaloptera (<1%), and Odonata (<1%). Non-insects found in samples included the freshwater crab (*Potamonautes perlatus*, Hydracarina, Planariidae, and Oligochaeta (all <1%). As well as the macroinvertebrates, Chydoridae occurred in quite high numbers during the warmer months of low flow, but these have not been included in the analyses of communities. We did, however, find *Chydorus* cf *piger* and *Alona* cf *rustica*, both of which were new records for Africa south of Sahel (D. G. Frey, Indiana University, Bloomington, IN, USA, pers. comm.).

The total number of species cannot be given, as many South African aquatic invertebrate groups have never been described to species, while some of those that have, such as the baetid Ephemeroptera, are now thought to be in need of extensive revision. We were able to sort, although not name, most taxa to species, and although the Diptera were sorted only to family, we considered the information adequate for classificatory purposes, as many researchers have commented on the usefulness of generic or familial data for categorising stream communities (see Furse et al. 1984 for discussion).

The local plecopteran nymphs are indistinguishable beyond genus and in Langrivier consisted almost exclusively of one or more species of the notonemourid genus *Aphanicerca*. Plecopteran nymphs of all sizes occurred throughout the year, forming a consistent 18–20% of the faunal numbers at different stations. Seven species of Ephemeroptera were present, four of which were Leptophlebiidae, two Baetidae, and one Ephemerellidae. *Castanophlebia calida* (8% overall) and the baetid *Acentrella capensis* (7%) were most common, and both increased in abundance downstream. Trichoptera were rare, with the petrothrincid (endemic family) *Petrothrincus triangularis* forming an overall 1% of the numbers but being virtually confined to L1, while the glossosomatid *Agapetus* sp was common only at L3 (3%). Coleoptera of the families Elmidae, Helodidae, Dryopidae, and Hydraenidae were common, especially in spring and summer, and at L1. Adults of a single hydraenid species contributed 4% to the total number of invertebrates (common only at L1), while an elmid larva contributed 3% (common at L1) and an helodid larva 6% (no downstream trends). Dipterans were represented mainly by Simuliidae (16%, no downstream trend), with probably four species of which *Simulium nigritarse* was most common, and by Chironomidae (15%, no trend). Blephariceridae (2%, and probably three species) were rare at L1 and more common downstream. All other Diptera each comprised <1% of total numbers. Dominance of the fauna by Diptera, Ephemeroptera, Plecoptera, and Coleoptera is typical of low-order streams of the region (Harrison and Elsworth 1958; Coetzer 1978; King 1983), with many species endemic to the fynbos biome.

Functional Feeding Groups

Analysis of gut contents of those animals contributing >3% of faunal numbers in Langrivier revealed an overwhelming dominance of very fine (<50 μm) organic material and virtually no green plant material. Other than the predators, the only animals with distinctly different gut contents were the Plecoptera, which had a high proportion of large particles consisting of sheets of connected plant cells. Even such relatively large particles, however, were seldom >35 μm, while all other taxa examined (mainly Leptophlebiidae, Baetidae, Ephemerellidae, Chironomidae, Blephariceridae, Simuliidae, Glossosomatidae, Dryopidae, Hydraenidae, and Helodidae) had guts containing detritus with particle sizes of about 5μm or less.

Allocation of animals to FFGs, using our information and separately using that of Merritt and Cummins (1984), provided results that in some cases differed quite markedly (Table 2). Shredders, collectors/gatherers, and scrapers were most affected because, using Merritt and Cummins' guidelines, a taxon was often apportioned between two or more groups, while using our information, it was assigned to a single group. Proportions obtained using the two methods were sometimes so different (e.g. L1, biomass for November) that they seemed barely related, suggesting that extrapolations from a text dealing with genera from another continent should be undertaken with caution. Of additional interest were the sometimes considerable differences in calculated proportions of FFGs at any one time along this short second-order stream, or when comparing data on numbers or biomass (Table 2). Further analyses of these data are deemed inappropriate, however, because of the reluctance with which we "forced" many taxa into the FFGs.

Difficulties were encountered mainly with the Plecoptera, Ephemeroptera, and Trichoptera (perhaps an irony, as we know these groups best). Our Plecoptera are small (<9 mm) and would be better described as skeletonisers than shredders, as they wear away the surface of a leaf, rather than chewing it. The trichopterans, *Petrothrincus* spp. and *Agapetus* sp., were described by Scott (1985) as grazers on rock surfaces, but their guts contained fine detritus and no algae, so describing them as scrapers (or herbivores, Merritt and Cummins 1984) seems

TABLE 2. Proportional assignment (percent) to functional feeding groups of Langrivier macroinvertebrates from three sites by numbers and by biomass. Two methods have been used: (A) using local information and (B) using information from Merritt and Cummins (1984). Results are presented for a month of peak leaf fall (November – late spring) and the first month of winter rains (May).

	Sites					
	L1		L2		L3	
FFG	A	B	A	B	A	B
	Percentage by numbers					
November						
Predators	4.62	5.30	4.75	4.75	3.63	3.63
Shredders	15.33	11.35	8.37	5.87	18.30	11.15
Collectors/gatherers	53.26	37.81	18.89	16.70	30.99	30.77
Filterers	14.18	14.18	55.34	55.34	24.19	24.19
Scrapers	7.88	26.67	8.43	13.08	20.08	27.45
Unclassified	4.73	4.69	4.22	4.26	2.81	2.81
May						
Predators	4.04	4.04	3.94	3.94	8.36	8.36
Shredders	23.03	15.01	26.49	16.90	15.67	10.59
Collectors/gatherers	46.67	43.33	40.11	36.98	28.75	26.72
Filterers	11.80	11.80	18.56	18.56	28.79	28.79
Scrapers	10.09	21.58	8.10	21.19	16.62	23.77
Unclassified	4.37	4.24	2.80	2.43	1.81	1.77
	Percentage by biomass					
November						
Predators	16.42	16.42	7.98	7.98	10.57	10.57
Shredders	20.37	11.84	12.29	7.07	25.20	13.57
Collectors/gatherers	41.39	31.84	18.93	17.92	27.73	31.34
Filterers	13.83	13.83	54.22	54.22	22.92	22.92
Scrapers	5.60	21.02	6.16	11.69	12.30	20.34
Unclassified	2.39	5.05	0.42	3.12	1.28	1.26
May						
Predators	17.73	17.73	7.67	7.67	13.12	13.12
Shredders	25.64	14.42	32.74	18.12	21.63	12.12
Collectors/gatherers	38.78	40.41	36.14	37.52	27.75	27.71
Filterers	7.96	7.96	18.37	18.37	27.70	27.70
Scrapers	8.49	18.10	4.65	17.90	8.57	15.14
Unclassified	1.40	1.38	0.43	0.42	1.23	4.21

an oversimplification. Both these and the baetids, and many more taxa, feed at least partly on the epilithon whether it contains green plant material or not.

The potential usefulness of the FFG concept is being hampered by functional groups and their definitions which do not adequately cater for all invertebrates, and by some fundamental problems with the concept itself. Since its inception by North American limnologists (for reviews, see Cummins 1973; Cummins and Klug 1979; Anderson and Sedell 1979; Lamberti and Moore 1984), for instance, several authors have noted differences in feeding habits and/or gut contents of a species during its life (e.g. Cummins 1973; Feminella and Stewart 1986) or between sites (Cummins 1973; Chessman 1986). This must bring into question the validity of using such groupings to examine the functional attributes of a benthic community, if the age structure of that community is not taken into consideration, or if results are extrapolated from one site to another. Sensitivity in detecting differences between streams/sites will also be reduced by sharing a species between two groups, and by assuming that a scraper, for instance, will be eating the same food in a sunny, nutrient-rich system, and a canopied nutrient-poor system. Confusion seems to have arisen largely because of the linking of feeding mechanisms and food.

Originally, the designation of FFGs stressed the feeding mechanisms of stream invertebrates rather than the food eaten (Cummins 1973; Cummins and Klug 1979). At the same time, however, an association was implied between shredders and coarse particulate organic matter (CPOM = >1000 μm) and between collectors and fine particulate organic matter (FPOM = 50–1000 μm). Cummins and Klug (1979), for instance, identified shredder/CPOM/fungal–bacterial "systems" and collector/FPOM–UPOM/bacterial "systems", while Bruns et al. (1982) recognised among their FFGs "CPOM shredders" and "FPOM gatherers."

A further precedent was set when the filipalpian Plecoptera became generally accepted as being "typical" shredders (e.g. Cummins 1973, 1974; Petersen and Cummins 1974; Cummins and Klug 1979), although Anderson and Sedell (1979) warned that some Plecoptera may not exhibit the chewing behaviour of typical shredders. Several authors have reported species of Plecoptera that feed in a similar way to ours, rasping or scraping the soft tissue from leaves (Wallace et al. 1970; Short et al.

1980), but they have usually concluded that these were still shredders (albeit microshredders), as their feeding caused particle size reduction and was thus important in the initial degradation of CPOM. Nevertheless, it has been noted that collectors such as some Ephemeroptera feed in a similar way, and on similar food (Anderson and Sedell 1979), while several Plecoptera appear to act more like collectors, browsing on surface accumulations of detritus and microorganisms (Chessman 1986).

Additional confusion has arisen because of the difficulty of observing the activities of small aquatic animals, which has often resulted in the assignation of FFGs being based on gut contents. The original proposals (that shredders, for instance, are those "with a general particle size range of food" of >1000 μm (Cummins 1973) or >4000 μm (Cummins 1974)) cannot readily be interpreted in terms of the size of particle within the animals' guts. Recognising this, Marchant et al. (1985) designated as shredders those invertebrates whose guts contained organic particles of size 50–1000 μm. On the other hand, Winterbourn et al. (1984) concluded that although 1–40% of the gut contents of their invertebrates consisted of particles in roughly the same size range (75–1000 μm), their streams contained no shredders.

We suggest that the term "shredder" needs redefinition so that it becomes clear whether or not it includes the small microshredders that skeletonise leaves, and if it does, how these animals can be distinguished from similarly feeding collectors. Clarity is also necessary on whether or not gut contents are useful guides to FFGs. More important is the fact that feeding adaptations, food particle size, and food quality are combined somewhat uneasily in the FFG concept and it seems necessary to decide whether it is the food or the feeders that are being categorised. As FFGs are purported to reflect stream ecosystem functioning, guidance on this might be obtained by deciding which is more significant for this functioning — the mode of feeding employed or the size or quality of the particle being attacked. If it is the mode of feeding, then shredders, for instance, may be animals fragmenting particles of any size, in which case they cannot be linked specifically with the degradation of CPOM and so the shredder:CPOM link would fall away. If it is the size of particles being eaten, then perhaps all those animals that feed (in a variety of ways) on any one size fraction of particle should be grouped together. If it is the quality of food, then useful distinctions could be made between, for instance, scrapers with algae in their guts and those with none, collectors that sweep fungi from leaves and those that eat sediments, and filterers that collect all small organic particles and those that select prey from constructed nets.

We appreciate and relate to the concept suggested by North American lotic ecologists, recognising the trends in food and feeding behaviour that they are striving to describe. Some southern African streams have taxa that are quite obviously shredders; our problem lies with the taxa that are less easily categorised. The situation bears similarities to that of two decades ago regarding the concept of trophic levels. The two concepts are attractive both in their simplicity and in the hypotheses that they generate. The concept of trophic levels faltered at least partly because of an inability to allocate many animals to a single category (i.e. trophic level) (Cousins 1985). We are now facing this problem with FFGs, possibly because of the inconsistencies in the literature. If the FFG concept is to be developed, then we feel that it needs adapting to increase its sensitivity and that the terms used should be more clearly defined, universally acceptable, and uniformly adhered to in order to be sure that perceived trends and differences are real. Until then, testing of the concept or using it for detailed comparisons of systems seems premature.

In our study, we can only conclude that the invertebrate fauna of streams such as Langrivier is dominated by detritivores feeding on allochthonous detritus. As almost all of the animals are very small, the particles that they ingest are also very small, although the Plecoptera ingest somewhat coarser particles than do the other invertebrates.

Analyses of Communities

Based on the mean root–root transformed abundance of 55 taxa recorded during the monthly sampling programme, and using the Bray-Curtis measure of similarity and group-average sorting, similar samples were identified in a series of analyses. Initially, the 16 samples collected randomly over the sampling site at L2 on a single day in October 1984 were analysed to look for patchiness or variation by microhabitat. Although collected in visually identified "runs," "riffles," and slow-flowing side areas, such perceived differences in habitat were slight and the faunal samples showed no obvious differences in species composition. When analysed with the rest of the samples from L2, those collected in October 1984 were scattered among those from the other spring months over the 3-yr period and showed no separation by habitat (Fig. 3). Comparison of the values for the environmental variables (BOM, water depth, current speed) measured for these 16 samples, on the basis of visually identified habitats, also showed no clear trends (King et al. 1987a), while Froude numbers (Fig. 3), which give an indication of turbulence (Statzner and Higler 1985), showed only a poorly defined trend from high to low numbers along the vertical axis. While acknowledging that techniques used in the important field of stream hydraulics (Gore 1978; Statzner and Higler 1986) would have helped clarify microdistribution patterns, we concluded that there were no major changes in faunal distributions within a site and that our three samples per site per time were adequately representative and could be further analysed.

To facilitate speed of processing, then, faunal samples collected each month were pooled by site, giving three composite samples per month. This was deemed acceptable because the main objective was to identify major temporal and spatial differences in community structure. One composite sample from L2 was poorly preserved and was thus discarded.

Analysis of the faunal samples taken from all three stations between April 1983 and May 1984 revealed a similarity at the 50% level for all but five samples (Fig. 4). The samples then split into a group of L1 samples and a group of mixed L2/L3 samples. The five outliers were two from L3, which were poorly preserved and thus represented incomplete data sets, and three dry-season samples from L1, which were the first group to split off in the dendrogram (at 42% similarity).

Using the same similarity matrix as above, MDS resulted in a two-dimensional plot displaying, through their distances apart, the relationship of samples to each other (Fig. 5). MDS had stress 0.387 in one dimension, 0.212 in two dimensions, and 0.156 in three dimensions, the comparatively small drop in stress between the two- and three-dimensional configurations indicating that the two-dimensional plot was a reasonable representation of the relationship between samples. This was confirmed by the grouping of samples on the plot, for L1 samples

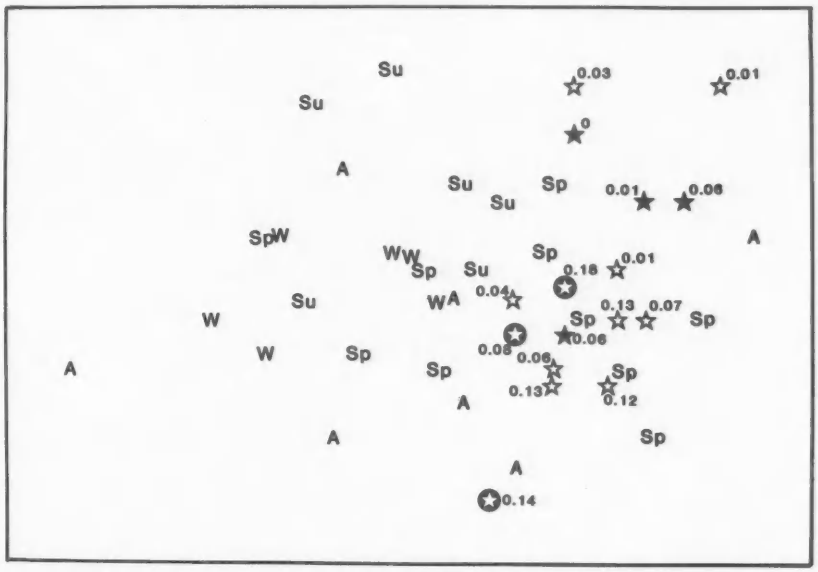

FIG. 3. MDS ordination of composite samples collected at L2 during three years, based on root–root transformed abundances of aquatic macroinvertebrates. Axis scales are arbitrary and are therefore not shown. The 16 single samples collected in October 1984 are identified by symbols: open star = run; solid star = slow, deep flow; circled star = riffle; Froude numbers are shown. All other samples are composite and are identified by season of collection: Sp = spring; Su = summer; A = autumn; W = winter.

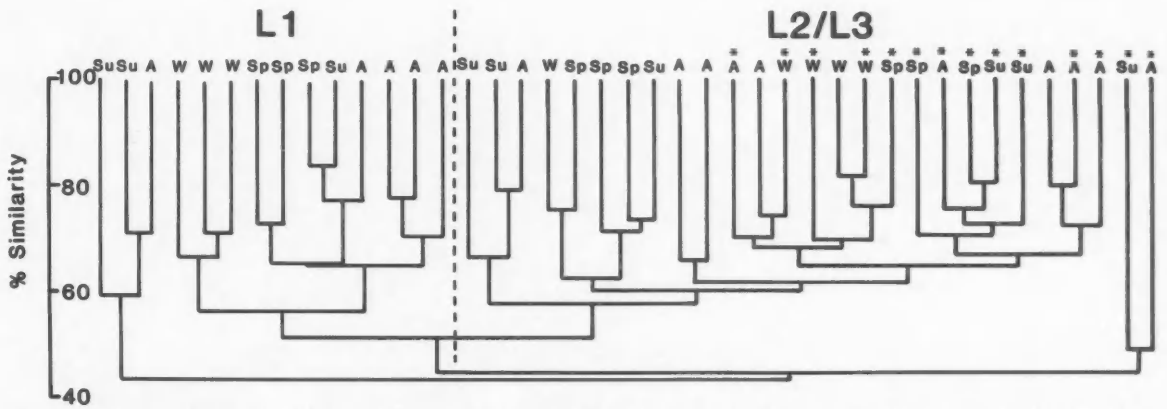

FIG. 4. Dendrogram showing classification of 41 composite faunal samples collected at three sites on Langrivier between April 1983 and May 1984. The two main biotic groups correlate with the upstream site (L1) and the two lower sites (L2 and L3). Seasons when samples were collected are indicated as in Fig. 3. Asterisks indicate samples from L3.

grouped separately from those of the two lower sites, with the same outliers indicated as in Fig. 4. Although the samples separated clearly by site, there was also some separation by season, with winter and summer samples at either extreme of both clusters (Fig. 5). The community composition remained basically the same at each site throughout the year, however, in contrast with the spatial pattern of two distinct communities in this second-order stream. A few of the species at L1 are newly recorded for the region or are very rare finds (of those seen by specialists to date this is true for the chydorids, for the simuliid *Prosimulium muspratti*, and possibly for the zygopteran) and obviously form part of a rare or rarely collected community. These species are restricted to the first kilometre or less of the stream.

Indicator Species

Information statistic tests revealed the taxa which were characteristic either of L1 or of L2/L3 (Table 3). There were no perfect indicators (taxa that were present in all samples of one group and in no samples of the other group), the best indicator species for L1 being dryopid adult A, which was present in all 14 samples from L1 but only 2 of the 23 samples from L2/L3 (samples from L2 and L3 in April 1984 and May 1984 were

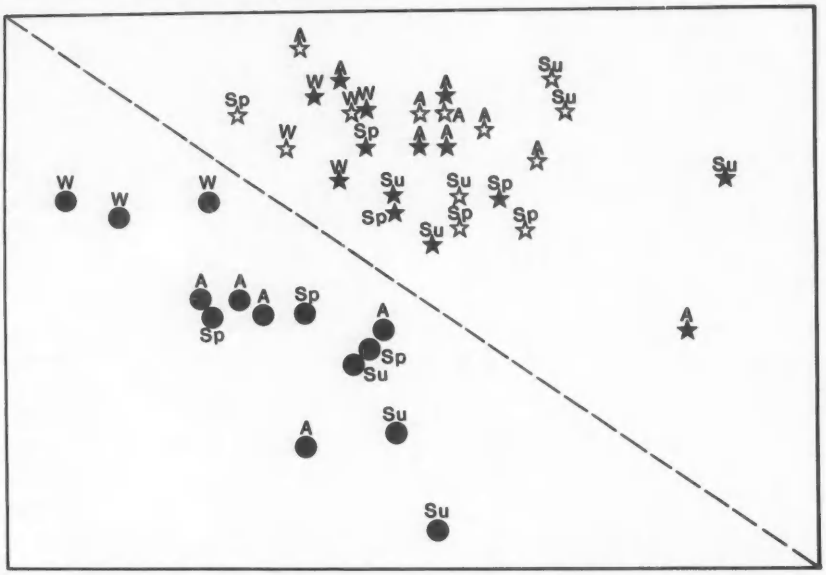

FIG. 5. MDS plot of the 41 samples classified in Fig. 4. Samples collected from L1 are identified by solid circles, those from L2 by open stars, and those from L3 by solid stars. The season of collection is identified as in Fig. 3.

TABLE 3. Frequencies of occurrence of indicator species, as identified by information statistics, which distinguish the biotic groups at (A) L1 (14 samples) and at (B) L2/L3 (23 samples). Scores for species differ at the 1 or 5% probability level.

Species	L1	L2/L3
(A) 1%		
Petrothrincus triangularis	11	2
Hydrosalpinx sericea	10	2
Dyschimus thrymmifer	4	0
Athericidae	14	9
Dryopid adult A	14	2
Zygopteran nymph A	9	1
5%		
Hydraenid adult B	9	7
Coleoptera adult 2	5	2
Coleoptera adult 4	2	0
Coleoptera adult 5	5	2
Elmid larva C	13	13
(B) 1%		
Baetis ?harrisoni	6	21
Chloroniella peringueyi	0	15
Chimarra sp. A	3	13
Chimarra sp. B	0	12
Cheumatopsyche spp.	3	19
Agapetus sp.	6	21
Parecnomina ?resina	0	11
Elmid larva A	0	13
5%		
Blephariceridae	4	15
Potamonautes perlatus	0	4

excluded from the analysis as the data base was too large, and these 2 mo represented an overlap of months; no samples were taken from L2 in July 1983). Other species strongly associated with L1 were *Petrothrincus triangularis, Hydrosalpinx sericea, Dyschimus thrymmifer, Atherix* sp., and an unidentified zygopteran (Table 3).

The best indicator species for L2/L3 was the megalopteran *Chloroniella peringueyi*, which occurred in 15 of 23 samples from L2/L3 but was never found at L1 (Table 3). Other invertebrates strongly associated with L2/L3 were five trichopteran species and elmid larva A. Separation of the three summer samples from L1 from the rest of that group was due to the total absence of *Lestagella penicillata* (Ephemerellidae), *Acentrella capensis*, and *P. triangularis* in those 3 mo, although they were abundant throughout the rest of the year.

Analysis of Environmental Data

Having identified different biotic groups along the stream, we searched for the environmental changes responsible using techniques described in the literature. Initially, data on the 23 environmental variables (King et al. 1987a; and unpubl. data) were arranged by month and site and then split into the groups L1 and L2/L3 as indicated by the faunal samples (Table 4). As L2 was not visited in July 1983, this month was excluded from the analysis for all three stations.

Many of the measured variables differed in value between the biotic groups at L1 and L2/L3; mean values of nine variables differed significantly (ANOVA, $P < 0.01$) between the two groups, and means for a further three variables differed at $P < 0.05$ (Table 4). The values for five of the variables whose means differed at $P < 0.01$ showed features which may be of biological importance. The highest value for potassium at L1 was 0.04 meq·L^{-1} (measured on four separate occasions), this being the same as the lowest value for L2/L3 (measured on only one occasion); the mean value of potassium at L2/L3 was 188% higher than at L1. Potassium has been mentioned before in stream studies as being an important factor influencing invertebrate distribution, particularly in pure mountain streams (Minshall

TABLE 4. Range and means (± SE) of environmental variables measured for the biotic groups L1 and L2/L3 as determined by one-way analysis of variance. Significant differences in means between the two biotic groups are shown. $*P < 0.05$, $**P < 0.01$, n.s. = not significant.

	L1		L2/L3		
Variable	Range	Mean	Range	Mean	Significance
Depth (mm)	140–500	252 ± 27	120–570	249 ± 19	n.s.
Current speed ($m \cdot s^{-1}$)	0.12–1.22	0.361 ± 0.079	0.30–1.56	0.543 ± 0.051	*
BOM ($g \cdot m^{-2}$)	5.64–68.10	22.325 ± 5.130	1.81–100.88	28.610 ± 4.728	n.s.
Temperature (°C)	7.5–15.1	10.87 ± 0.70	7.00–20.0	13.07 ± 0.62	*
Dissolved oxygen (%)	93–108	99.3 ± 1.0	91–109	99.5 ± 0.7	n.s
TDS ($mg \cdot L^{-1}$)	7.3–55.3	26.37 ± 3.21	19.0–47.3	32.49 ± 1.65	n.s.
Conductivity ($mS \cdot m^{-1}$)	1.70–2.40	1.992 ± 0.065	1.97–3.44	2.593 ± 0.067	**
Sodium ($meq \cdot L^{-1}$)	0.09–0.22	0.162 ± 0.010	0.17–0.30	0.230 ± 0.006	**
Potassium ($meq \cdot L^{-1}$)	0.00–0.04	0.025 ± 0.003	0.04–0.10	0.072 ± 0.015	**
Calcium ($meq \cdot L^{-1}$)	0.002–0.255	0.045 ± 0.025	0.001–1.050	0.095 ± 0.043	n.s.
Magnesium ($meq \cdot L^{-1}$)	0.020–1.486	0.147 ± 0.112	0.026–4.444	0.453 ± 0.215	n.s.
Chloride ($meq \cdot L^{-1}$)	0.06–0.09	0.083 ± 0.002	0.08–0.12	0.101 ± 0.001	**
Sulphate ($meq \cdot L^{-1}$)	0.01–0.08	0.038 ± 0.006	0.02–0.30	0.104 ± 0.012	**
Silicate ($\mu eq\ Si \cdot L^{-1}$)	0.14–0.60	0.382 ± 0.052	0.21–2.98	1.127 ± 0.159	**
Nitrite ($\mu eq\ N \cdot L^{-1}$)	0.03–0.14	0.079 ± 0.009	0.08–0.26	0.129 ± 0.009	**
Nitrate ($\mu eq\ N \cdot L^{-1}$)	0.70–8.66	2.725 ± 0.566	1.03–10.34	4.046 ± 0.487	n.s.
Phosphate ($\mu eq\ P \cdot L^{-1}$)	0.05–0.50	0.263 ± 0.042	0.01–0.54	0.199 ± 0.030	n.s.
Ammonia ($\mu eq\ N \cdot L^{-1}$)	2.10–9.22	4.222 ± 0.629	2.29–7.68	4.342 ± 0.290	n.s.
pH	4.3–5.6	4.95 ± 0.11	5.2–6.8	6.00 ± 0.10	**
H^+ ($meq \cdot L^{-1}$)	0.000–0.050	0.015 ± 0.005	0.000–0.006	0.002 ± 0.000	**
Alkalinity ($meq \cdot L^{-1}$)	0.00–0.15	0.060 ± 0.014	0.00–0.19	0.095 ± 0.010	*
Colour (Hazen units)	3.0–35.0	9.3 ± 2.3	0.00–25.0	7.1 ± 1.2	n.s.
Polyphenols ($mg \cdot L^{-1}$)	0.09–0.52	0.208 ± 0.033	0.05–0.63	0.169 ± 0.023	n.s.

TABLE 5. Significant ($P < 0.01$) positive (+) and negative (−) correlations between environmental variables.

	Depth	Current speed	BOM	TDS	Conductivity	Temperature	Colour	Alkalinity	pH	Polyphenols	Dissolved oxygen	Sodium	Potassium	Calcium	Magnesium	Ammonia	Chloride	Sulphate	Silicate	Nitrite	Nitrate	Phosphate	H^+
Depth																							
Current speed	+																						
BOM																							
TDS		+																					
Conductivity																							
Temperature	−		+																				
Colour	+	+																					
Alkalinity			−																				
pH					+	+																	
Polyphenols							+	−															
Dissolved oxygen																							
Sodium					+	+			+														
Potassium					+	+			+			+											
Calcium																							
Magnesium														+									
Ammonia																							
Chloride					+	+	−		+			+	+										
Sulphate			+			+			+			+	+				+						
Silicate												+	+				+						
Nitrite					+							+	+	+	+		+						
Nitrate			−		+								+						+				
Phosphate	+		−			−																	
H^+					−				−			−	−				−						

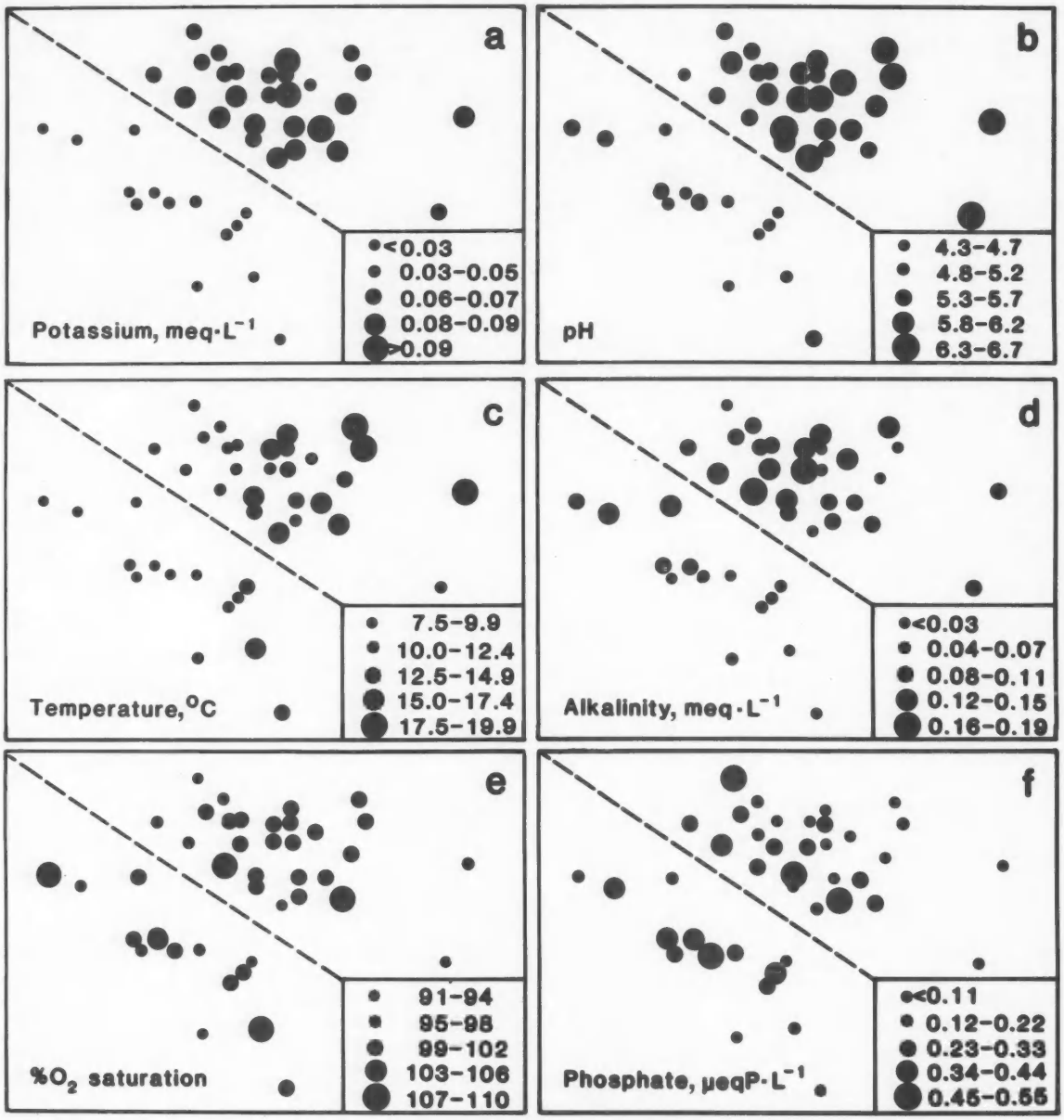

FIG. 6. Relationship of the biotic groups to environmental conditions. The MDS plot of faunal samples collected at the three sites (Fig. 5) forms a template upon which values of single environmental variables are entered. Samples from L1 are at the bottom left of each plot, and those from L2/L3 are at the top right. Variables illustrated (a–f) show different degrees of separation of values between the groups.

and Minshall 1978; Bunn et al. 1986). As it is a major nutrient and is often present in very small amounts, it is likely that potassium is a limiting factor at L1. It is also possible, however, that because the levels of this ion are near the lower limit of detectability, the apparent differences in concentration are artificially inflated.

Chloride showed only a small overlap of values between the two groups, but the mean at L2/L3 was only 22% higher than at L1, much less than the difference for potassium. The maximum values of sulphate and silicate at L1 were very low compared with those at L2/L3, while the maximum value of H^+ at L2/L3 was very low compared with that at L1. The significance to the fauna of these differences, if any, is not apparent. Of the other variables whose means differed significantly ($P < 0.01$) between groups (pH, sodium, conductivity, and nitrite), only pH is known to be correlated with invertebrate distribution patterns (Harrison and Agnew 1962; Minshall and Minshall 1978; Townsend et al. 1983). Correlation also existed between many

of the variables (Table 5). For example, three of the variables whose means differed significantly ($P < 0.01$) between biotic groups (potassium, sodium, and chloride) correlated strongly not only with the biotic groups ($r > 0.70$ in each case) but also with each other ($r > 0.74$ in each case).

Stepwise discriminant analysis and multiple linear regression identified certain variables as having significantly different values between biotic groups, but the variables identified as important depended on which were used in the analyses. Stepwise discriminant analysis produced good separation of groups, but when repeated with random different combinations of variables omitted, satisfactory groupings were again obtained, although with different variables entered into the classification function. Similarly, highly significant multiple linear regression was obtained (with biotic group as the dependent variable) with low residual sum of squares. When repeated with different combinations of variables omitted, however, satisfactory regressions were again obtained although with different variables having significant regression coefficients. For example, when all 23 environmental variables were included, none had a significant regression coefficient. When only conductivity, sodium, potassium, chloride, and phosphate were included, however, all except chloride had significant regression coefficients.

We believe that these unstable results were due to the strong correlations that existed between various environmental variables (Table 5) and to the similarity between the number of samples (39) and the number of variables (23) used in the analyses (i.e. the low degrees of freedom). Because of the strong correlations, some variables may have been unduly affected by the inclusion or exclusion of other variables in the analyses, and so it was difficult to determine the importance of any one to faunal distributions.

We therefore further investigated correlations between faunal clusters and the environmental variables using the MDS plot of faunal samples (Fig. 5) as a template upon which were superimposed, one at a time, values for the measured environmental variables. This allowed a visual assessment of the correlations both between the two biotic groups and each environmental variable, and between variables. Distribution of values of individual variables among the two biotic groups ranged from good separation of high and low values between the groups to no clear pattern of separation (Fig. 6). Generally, there was good to moderate separation for those variables with mean values that differed significantly ($P < 0.01$) between groups. Variables with mean values that did not differ significantly ($P > 0.05$) between groups, however, showed no clear separation of values on the MDS plot. Potassium, for instance, showed an almost complete separation of values into the two groups (Fig. 6) and pH a reasonably clear separation with some overlap (both with means differing significantly at $P < 0.01$). Temperature and alkalinity ($P < 0.05$) showed poor separation between groups, however, and dissolved oxygen and soluble reactive phosphate ($P > 0.05$) showed none (Fig. 6). This supports our finding that, for this stream, significant differences in mean annual values of an environmental variable between biotic groups are good indicators of which variables correlate with the distribution of those groups.

Although providing a guide to variables that are correlated with community distribution patterns, none of the analytical techniques used gives an indication of which of the variables measured, if any, are primary determinants of distribution, and which are merely correlated with that distribution. Many stream ecologists believe that the combined effects of subtle differences in several variables may have a greater effect on biotic distributions than a single variable with a distinct pattern of values. In retrospect, we feel that the above techniques may well reveal valid environmental differences between substantially different communities, but that the more subtle differences occurring between similar communities should be traced in other ways. A possible approach could be to record the conditions in which selected species occur throughout their geographical ranges and to test perceived trends through carefully planned laboratory experiments. This approach is relatively time consuming, but in the long run should provide more accurate and biologically useful results than computer analyses which are designed to identify statistical rather than ecological differences.

Comparative Studies: Spatial and Temporal Scales

Worldwide, a long list of variables has now been shown to correlate with faunal distribution patterns (Cushing et al. 1983; Townsend et al. 1983; Furse et al. 1984; Wright et al. 1984; Marchant et al. 1985; Bunn et al. 1986). At present, such a wealth of information is confusing because there is no uniformity in the results, and thus no guarantee that even at the coarser scale, variables shown as being correlated or not correlated with biotic distributions in one river (or group of rivers) are so in other rivers. Low levels of aluminium, for instance, might be unimportant, in terms of the effect on the biota, in an alkaline stream, but exceedingly important in an acid stream.

It thus seems timely to see if it is possible to determine the types, and level of resolution, of biotic and environmental data that should be used for a particular kind of study. At present the full spectrum, from external geographic variables (i.e. driving variables, see Lotspeich 1980) to instream (or passive, see Lotspeich 1980) variables, has been included in different studies. According to Lotspeich (1980), driving variables are related to the major natural forces that shape the character of river ecosystems (e.g. bedrock geology, geomorphology, climate, and hydrology), while the passive variables result from the combined influences of all the higher controlling forces. Passive variables are usually related to the river itself (e.g. water chemistry and substratum composition) and play a more passive role in river ecosystem functioning in that they cannot influence the higher controlling forces. Driving forces listed in various studies as correlating with biotic distributions include altitude, position on river, gradient, and current speed, while the passive variables include those relating to water quality, substratum, and allochthonous detritus.

We feel that the inclusion of both driving and passive variables in a single analysis could give unreliable results. This is because the driving variables that are necessary to distinguish rivers or river zones on a large scale could cloud the issue on a small scale, masking subtle but important instream differences. In other words, the choice of variables used in an analysis of very different streams would probably not be suitable for comparing similar streams, and vice versa. Additionally, it must generally be true that the more similar two (or more) rivers are, the more variables there will be that do not vary significantly between them. Thus it should be possible to judge, in some logical fashion, which variables to include or exclude from a specific study.

Initially, for instance, the driving variables and a coarse classification of the biota could be used to group streams in different biomes (or biogeographic regions). If a more detailed under-

standing of the distribution of the fauna was required, then, at a finer resolution, relationships between the instream variables and the aquatic biota could be sought for each group separately.

In effect this means predetermining to some extent the environmental limits within which a type of stream occurs. This could be achieved through a hierarchical classification structure (see Frissell et al. 1986), perhaps using biogeoclimatic region, vegetation and/or soil types, and river zone as the first three levels. At each level of the hierarchy the biota could be used as a guide and environmental limits set which would define the limits of, and narrow the sets of variables needed for, the lower levels. Using this method, mountain streams of the southwestern Cape of South Africa, for instance, should group together and occur within specified limits of precipitation, temperature, altitude, geological formations, vegetation types, and so on and have broadly similar biota. Each of these features would occur at one level of the classification and be excluded from subsequent levels; they could, however, be reintroduced at a lower level if information at a finer resolution were needed.

Rivers grouping together at the zonal level could then be dealt with as a group, using suitable techniques to identify both subgroups within the biota and the instream variables that might be influencing biotic distributions. Such an exercise should allow identification of (a) streams with a similar ecological functioning so that extrapolations may be made between them for management purposes and (b) variables that differ subtly between streams grouped under different but closely related biotic subgroups and which could be influencing the faunal distributions.

Such an approach to river classification is at the conceptual stage (South African Council for Scientific and Industrial Research, Working Group on River Classification) and shares features with several other classificatory systems proposed for rivers that are also based on numerical analyses (see, for example, Cushing et al. 1983; Wright et al. 1984). If it could be implemented successfully, it might, for instance, help to circumvent the problem noted by Cushing et al. (1983) that streams of the same order in different biomes are not similar. Here, biomes would be separated in the hierarchical classification.

Seasonal Changes in Invertebrate Communities

Our data show that no major temporal changes in invertebrate community composition occur in Langrivier. The presence at each of our sampling sites of a single community throughout the year is a phenomenon also recorded for the headwaters of the Eerste River (King 1981), where it was attributed to the slow growth of species in these oligotrophic waters. Within 5 km downstream of the confluence of the Eerste River and Langrivier the situation changes to the distinct summer and winter communities so characteristic of all zones of southwestern Cape streams other than their extreme headwaters (King 1981).

Seasonal changes in the composition of stream invertebrate communities in the Southern Hemisphere have been considered by several authors recently (Winterbourn et al. 1981; Lake et al. 1985a; Hart 1985; Bunn et al. 1986). It has been suggested that the faunas of Australian and New Zealand streams are more weakly seasonal, with flexible, less synchronous life cycles than their North American counterparts (see Lake et al. 1985a for references). This has been attributed to, *inter alia*, the high variability and unpredictability of discharge in the southern rivers (Winterbourn et al. 1981). Bunn et al. (1986), however, reported marked seasonal changes in the fauna of streams in southwestern Australia and suggested that these may be correlated with the better defined and more predictable seasonal changes in the physical and chemical conditions of the streams in this mediterranean ecosystem than in rivers in the rest of Australia.

Southern African streams show similar differences in predictability of streamflow. At one extreme, streams in the southwestern Cape exhibit regular seasonal changes in discharge that correlate with predictable dry and wet seasons. At the other extreme, are rivers such as the Hartbeesrivier, in arid central South Africa, with an annual runoff that in one year was 1700% of the annual average, in another year was only 9% of the annual average, and in 1961 alone was more than the total in the previous 27 yr of record (Cambray et al. 1986). In this context, there are greater similarities in patterns and predictability of flow between streams in widely separated mediterranean ecosystems (southwestern Australia, Bunn et al. 1986; southwestern South Africa, King 1981; southern California, Cooper et al. 1986) than between those across the southern African subcontinent. A common feature of most zones of streams in these three mediterranean ecosystems is the predictable occurrence of distinctive winter and summer invertebrate communities, with a rapid transition from summer to winter communities at the onset of winter rains, and a more gradual return to the summer communities at the start of summer.

This is in stark contrast with the situation reported for eastern Australia and New Zealand (Winterbourn et al. 1981) and the eastern Cape of South Africa (F. de Moor, Albany Museum, Grahamstown, R.S.A., pers. comm.), where flow is less predictable and seasonality of the aquatic invertebrates is not distinct. Such a phenomenon should not be confused with the situation regarding communities in headwaters such as Langrivier, where there are no seasonal changes in species but predictable seasonal changes in numbers. It seems that the lack of more obvious seasonal changes in community structure in Langrivier is because of slow growth in the oligotrophic waters, whereas the lack of seasonality in rivers in eastern Australia and eastern South Africa is due to unpredictability of flow. Clearly, comparative research on the streams in mediterranean ecosystems would be a productive line of research, while country-wide generalisations on stream–ecosystem functioning, both in Australia (as concluded by Bunn et al. 1986) and in South Africa, could be less useful.

Acknowledgements

We acknowledge the contributions to this work of Barbara Byren and Pat Hawker, who assisted in the collecting and sorting of faunal samples, and of Coleen Moloney and Patti Wickens, who helped with the early stages of the numerical analyses. We thank Dr. F. C. de Moor and Dr. K. M. F. Scott for their identifications of trichopteran and simuliid species, Dr. D. G. Frey for his identification of the Chydoridae, and Miss J. Graves for analysing gut contents. The Forestry Branch of the Department of Environmental Affairs allowed us to work in the Jonkershoek State Forest and provided data on stream discharge and rainfall. The study was financed by the Fynbos Biome Project of the Council for Scientific and Industrial Research. We also thank J. A. Gore, J. V. Ward, and an anonymous referee for valuable criticisms of the manuscript; the ideas contained in this paper do not necessarily reflect their views.

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Gustatory Responses to Tetrodotoxin and Saxitoxin in Fish: A Possible Mechanism for Avoiding Marine Toxins

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Yamamori, K., M. Nakamura, T. Matsui, and T. J. Hara. 1988. Gustatory responses to tetrodotoxin and saxitoxin in fish: a possible mechanism for avoiding marine toxins. Can. J. Fish. Aquat. Sci. 45: 2182–2186.

The gustatory responses to tetrodotoxin (TTX) and saxitoxin (STX) recorded from the palatine nerve (VIIth cranial nerve) were studied electrophysiologically in rainbow trout (*Salmo gairdneri*) and Arctic char (*Salvelinus alpinus*). Both toxins were highly effective gustatory stimuli in both species. In rainbow trout, TTX had a threshold concentration 2×10^{-7} mol/L and at 10^{-5} mol/L evoked a response four times that of 10^{-3} mol L-proline/L, the most potent amino acid for this species. The threshold for STX was lower (10^{-8} mol/L), but unlike TTX the response magnitude reached a maximum at 10^{-6} mol/L. The reverse occurred in Arctic char; lower threshold for TTX (10^{-8} mol/L) than STX (10^{-7} mol/L) and the response magnitude never exceeded that of 10^{-3} mol L-proline/L. Cross-adaptation experiments indicated that the receptor(s) for TTX are distinct from those which detect amino acids and bile salts and that TTX and STX do not share the same receptor populations. Furthermore, the integrated response to TTX or STX was a fast-adapting, phasic response and rapidly returned to baseline even with continued stimulation. Perfusion of the gustatory organs with these toxins had little toxic effect. The sensitive, specific gustatory receptor system for the toxins suggests the existence of a mechanism for avoiding poisonous prey organisms that has adaptive advantage to the receiver (predator).

Les réponses gustatives à la tétrodotoxine (TTX) et à la saxitoxine (STX) enregistrées à partir du nerf palatin (VII[e] nerf crânien) ont été étudiées électrophysiologiquement chez la truite arc-en-ciel (*Salmo gairdneri*) et chez l'omble de l'arctique (*Salvelinus alpinus*). Ces deux toxines sont des stimulis gustatifs très efficaces chez les deux espèces. Chez la truite arc-en-ciel, la TTX avait une concentration seuil de 2×10^{-7} mol/L et à 10^{-5} mol/L, elle provoquait une réponse quatre fois supérieure à celle de la L-proline à 10^{-3} mol/l, l'acide aminé dont l'effet est le plus puissant pour cette espèce. Le seuil pour la STX était inférieur (10^{-8} mol/L), mais contrairement à ce qu'on a observé pour la TTX, la réponse a atteint un maximum à 10^{-6} mol/L. On a observé le phénomène inverse chez l'omble de l'arctique, soit un seuil inférieur pour la TTX (10^{-8} mol/L) et avec la STX (10^{-7} mol/L), et l'importance des réponses ne dépassait jamais celle de la L-proline à 10^{-3} mol/L. Les expériences d'adaptation croisée ont indiqué que le ou les récepteurs de la TTX sont différents de ceux qui détectent les acides aminés et sels biliaires, et que la TTX et la STX ne partagent pas les mêmes populations de récepteurs. En outre, la réponse intégrée à la TTX ou à la STX était une réponse en phase à adaptation rapide, avec un retour rapide à la ligne de base, même avec une stimulation continue. La perfusion des organes gustatifs avec ces toxines avait peu d'effets toxiques. Le système de récepteur gustatif particulier et sensible pour ces toxines suggère l'existence d'un mécanisme permettant d'éviter des proies toxiques, qui constitue un avantage d'adaptation pour le récepteur (prédateur).

Received November 17, 1987
Accepted July 26, 1988
(J9487)

Reçu le 17 novembre 1987
Accepté le 26 juillet 1988

Puffer toxin, tetrodotoxin (TTX), is one of the most potent neurotoxins known, with its lethal toxicity to humans 300 times that of cyanide. TTX exerts its toxic action by specifically blocking the voltage-sensitive sodium channels in nerve and muscle membranes (Narahashi 1974; Kao 1981). TTX was once believed to be found exclusively in tetraodontid fishes, 16 species altogether including 12 species of the genus *Fugu* (Tani 1945), but its distribution seems much wider than originally thought. It has been shown to be present in a variety of aquatic organisms: California newt (*Tarica torosa*) (Buchwald et al. 1964), goby (*Gobius criniger*) (Noguchi and Hashimoto 1973), Costa Rican atelopid frogs (*Atelopus* sp.) (Kim et al. 1975), blue-ringed octopus (*Octopus maculosus*) (Scheumack et al. 1978), Japanese ivory shell (*Babylonia japonica*) (Noguchi et al. 1981; Yasumoto et al. 1981), trumpet shell (*Charonia sauliae*) (Narita et al. 1981), Japanese starfish (*Astropecten polyacanthus*) (Noguchi et al. 1982), xanthid crab (*Atergatis floridus*) (Noguchi et al. 1983), frog shell (*Tutufa*

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lissostoma) (Noguchi et al. 1984), and flatworms (Jeon et al. 1986). Whether TTX in puffers is synthesized endogenously or accumulated exogenously through the food chain is not clearly understood, but the finding that puffers cultured with artificial foods become nontoxic suggests an exogenous origin (Matsui et al. 1981, 1982). Isolation of TTX-producing bacteria *Vibrio* sp. from the intestinal flora of the xanthid crab further supports this view (Noguchi et al. 1986).

Puffers, when stimulated by handling or electrical shock, secrete TTX into the surrounding water from a unique gland-like structure in the skin containing a high concentration of TTX (Kodama et al. 1986). This response is usually accompanied by swelling of the abdomen (Kodama et al. 1985; Saito et al. 1985). TTX has been implicated in repelling predators. In addition, preliminary behavioural studies demonstrated that some fish species (rainbow trout, striped beak perch, greenlings, etc.) reject toxic puffer livers and artificial food pellets containing TTX (Yamamori et al. 1980). These findings suggest that fish may detect TTX via the gustatory system and thereby protect themselves from poisonous prey.

Although earlier studies have shown that TTX did not induce electrical responses in the gustatory systems of frog and rat (Ozeki and Noma 1972), no investigation has been reported in fish. The present study was designed to investigate the gustatory sensitivity of rainbow trout (*Salmo gairdneri*) and Arctic char (*Salvelinus alpinus*) to TTX by measuring the integrated electrical responses from the palatine nerve innervating the palate and inside the upper lip. These species were chosen primarily because of laboratory convenience; however, they are both anadromous and could encounter marine toxins in nature. Furthermore, the gustatory sensitivity and specificity for amino acids and bile salts have been well investigated in both species (Marui et al. 1983; Sveinsson 1985). Saxitoxin (STX), a paralytic shellfish toxin having similar pharmacological actions to TTX, was also tested. STX is believed to be only one of 10–12 closely related naturally occurring toxins produced by dinoflagellates of the genus *Gonyaulax*; a massive bloom causes the phenomenon known as "red tide" in coastal waters.

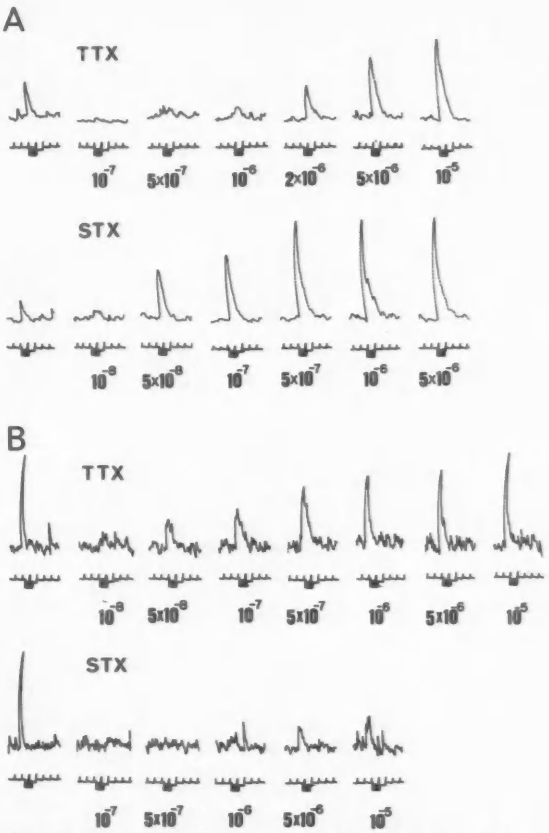

FIG. 1. Integrated gustatory responses to increasing concentrations of tetrodotoxin (TTX) and saxitoxin (STX) in (A) rainbow trout and (B) Arctic char. Tracings at the far left in each column represent the response to the standard stimulus, 10^{-3} mol L-proline/L. Concentrations are shown in mol/L. Time signal, each division = 5 s.

Materials and Methods

Rainbow trout and Arctic char, 20–27 cm in body length, were obtained from the Rockwood Hatchery, Freshwater Institute. Fish were held in fiberglass tanks supplied with dechlorinated Winnipeg city water (water temperature 11.5–12.5°C).

Gustatory responses were recorded from palatine nerves (VIIth cranial nerve) innervating palatal taste buds and inside the upper lip, using the method of Marui et al. (1983). Briefly, the fish were paralyzed with an intramuscular injection of Flaxedil (gallamine triethiodide, 5 mg/kg body weight) and positioned in a plexiglass trough with dechlorinated water perfusing the gills throughout the experiments. The eyeball was removed to expose a nerve branch running through the bottom of the eye socket to the oral roof. The freed nerve bundle was severed centrally and its peripheral end was placed on bipolar platinum–iridium electrodes. Mineral oil was added to the orbit to prevent drying of the nerve preparation during recording. The electrical activity of the whole nerve bundle was amplified (Grass 7P511), integrated (Grass 7P3; time constant 0.5 s), and recorded on a polygraph (Grass 7B). The response magnitude was measured as the height of integrated responses over the stimulus duration of 5 s and expressed as a percentage of a response to a standard stimulus, 10^{-3} mol L-proline/L. Each stimulus was tested twice consecutively, with a standard time interval of 2 min, and the mean responses were obtained. For delivering chemical stimuli, an apparatus described previously was used (Marui et al. 1983; Evans and Hara 1985).

In cross-adaptation experiments, the gustatory organs were perfused with 10^{-3} mol L-proline/L, 10^{-3} mol L-α-amino-β-guanidinopropionic acid (AGPA)/L, 10^{-3} mol betaine/L, or 10^{-5} mol taurolithocholic acid (TLCA)/L, and a concentration series of TTX, dissolved in respective adapting stimulant solutions, were tested. Response magnitudes were compared with those prior to adaptation (control). In the second series of cross-adaptation experiments, the receptors were adapted to 10^{-6} or 5×10^{-6} TTX/L and responses to varied concentrations of STX were tested. The reverse were also examined.

Stock solutions (10^{-3} mol/L) of TTX and STX were made with distilled water and stored in a refrigerator. TTX (3.19 mg) was initially dissolved in 0.1 mL of 6% acetic acid solution to which distilled water was added to obtain the final stock concentration. All final test solutions were freshly prepared, just before each application, with the same water used to perfuse the gills and palate. The pH of these test solutions ranged from 7.3 to 7.7, and therefore no adjustment was made. TTX was donated by Sankyo Co., Ltd. (Tokyo). STX (diacetate form) and AGPA were purchased from Calbiochem-Behring Corp.

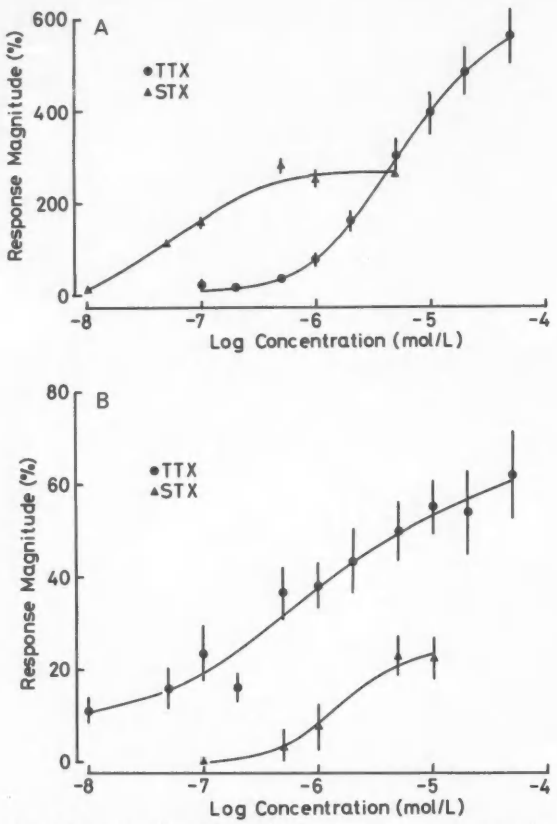

FIG. 2. Semi-logarithmic plots of the concentration–response relationships to tetrodotoxin (TTX) and saxitoxin (STX) in (A) rainbow trout and (B) Arctic char. Average response magnitude is represented as a percentage of that induced by the standard stimulant, 10^{-3} mol L-proline/L. Points represent mean ± SE of 7–14 fish.

(La Jolla, CA), and TLCA and other amino acids were from Sigma Chemical Co. (St. Louis, MO). All chemicals were of the highest purity available from commercial sources.

Results

Response Characteristics to TTX and STX

Typical gustatory responses of rainbow trout and Arctic char to increasing concentrations of TTX and STX are presented in Fig. 1. The integrated response to TTX and STX, a fast-adapting and phasic response, rapidly returned to the baseline even with continued stimulation. The concentration–response (C–R) relationships were generally sigmoidal, when plotted semilogarithmically (Fig. 2). In rainbow trout, a clear response to TTX appeared at 2×10^{-7} mol/L and the magnitude reached six times that of the standard 10^{-3} mol L-proline/L response at 5×10^{-5} mol/L. The threshold for STX was lower, 10^{-8} mol/L. Unlike TTX the response magnitude reached a maximum at 5×10^{-7} mol/L and no further increase occurred with higher concentrations (saturation; Fig. 2A). In Arctic char, the threshold concentration for TTX was lower than that for STX (10^{-8} versus 10^{-7} mol/L). However, the general shapes of C–R relationships were similar to those observed in rainbow trout (Fig. 2B). Because the response magnitude to 10^{-3} mol L-proline/L was greater in Arctic char than in rainbow trout, the relative response magnitudes to both TTX and STX appeared smaller, never exceeding that of the standard (Fig. 2B).

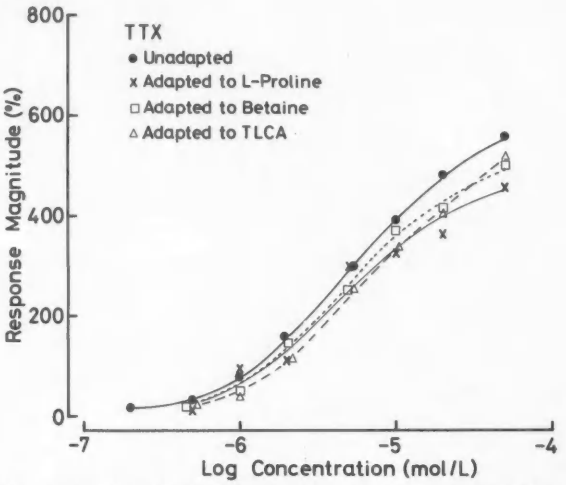

FIG. 3. Semi-logarithmic plots of the concentration–response relationships to tetrodotoxin (TTX) before (●) and during adaptation to 10^{-3} mol L-proline/L (×), 10^{-3} mol betaine/L (□), and 10^{-5} mol taurolithocholic acid (TLCA)/L (Δ). Average ($N = 3$) response magnitude is represented as a percentage of that induced by the standard stimulus, 10^{-3} mol L-proline/L.

Cross-adaptation

To evaluate whether there are different types of gustatory receptors, or transduction processes for TTX (and STX) and other known gustatory stimuli (amino acids and bile salts), cross-adaptation experiments were performed. If two stimuli share the same receptors, gustatory responses will be reduced when the receptors are adapted to one of the stimuli. Adaptation to 10^{-3} mol L-proline/L, 10^{-3} mol betaine/L, or 10^{-5} mol TLCA/L had little effect on responsiveness to TTX in rainbow trout (Fig. 3). The responses elicited by TTX during L-proline or TLCA adaptation were slightly reduced, but the reduction was not significant. Similarly, in Arctic char, adaptation to L-proline, which elicited greater responses than TTX at all concentrations (cf. Fig. 2B), had no significant effect on the TTX response (data not presented). Conversely, adaptation to 5×10^{-6} mol TTX/L partially inhibited L-proline responses (60 ± 35% SD), but had little effect on responses to 10^{-3} mol AGPA/L (90 ± 16% SD) and 10^{-7} mol/L TLCA/L (82 ± 22% SD).

In rainbow trout, adaptation to 10^{-7} mol·L^{-1} STX had little effect on TTX responses (Fig. 4A). Adaptation to 10^{-6} mol TTX/L had no effect on STX responses at concentrations between 10^{-8} and 5×10^{-7} mol/L (Fig. 4B). Perfusion with 5×10^{-6} mol TTX/L had no effect on STX response at 5×10^{-6} mol/L (99 ± 30% SD), at which both chemicals elicited approximately equal response magnitudes.

Discussion

The extreme gustatory sensitivity of the trout and char to the marine toxins TTX and STX corroborates our earlier behav-

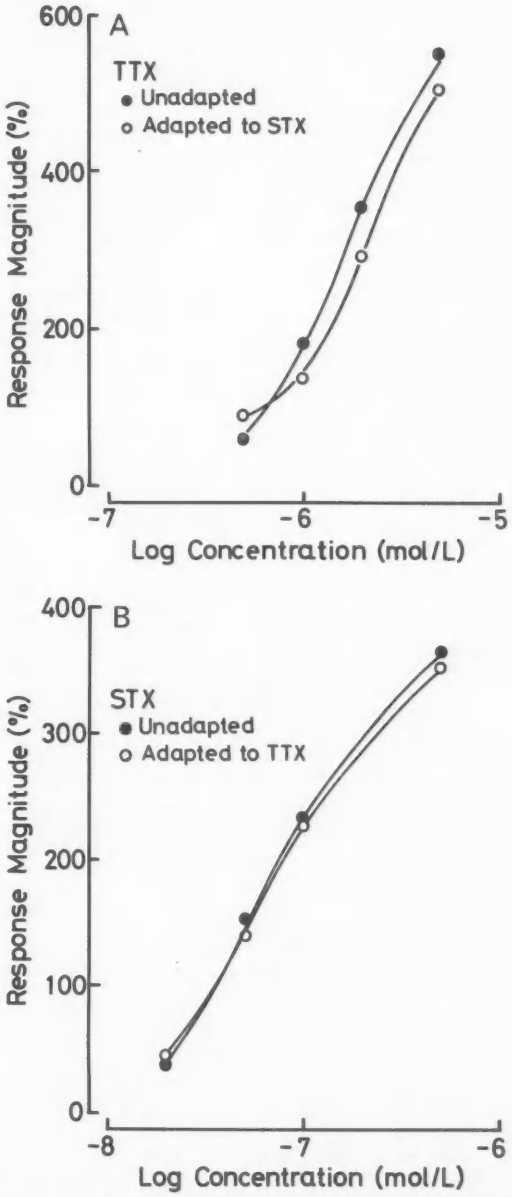

FIG. 4. (A) Semi-logarithmic plots of the concentration-response relationships to tetrodotoxin (TTX) before (●) and during (○) adaptation to 10^{-7} mol saxitoxin (STX)/L. (B) Semi-logarithmic plots of the concentration-response relationships to saxitoxin (STX) before (●) and during (○) adaptation to 10^{-6} mol tetrodotoxin (TTX)/L. In both graphs, average ($N = 3$) response magnitude is represented as a percentage of that induced by the standard stimulus, 10^{-3} mol L-proline/L.

ioural findings that fishes detect these toxins through the gustatory system and the suggestion that they might protect themselves from poisonous prey (Yamamori et al. 1980). The response obtained is fast adapting and phasic, similar to the gustatory amino acid responses (Marui et al. 1983; Sveinsson 1985). The estimated threshold concentrations of the toxins in the present study are comparable with that of L-proline, the most stimulatory amino acid for rainbow trout (Marui et al. 1983). A somewhat lower threshold value (10^{-11} mol/L) has been known for TLCA (Hara et al. 1984). This is the first study to demonstrate that TTX and STX stimulate the gustatory organ of fish. In contrast, TTX even at 3.08×10^{-6} mol/L did not stimulate the gustatory systems (receptor cells and chorda tympani nerve fibers) of frog and rat, nor did it inhibit the generation of receptor potential in response to gustatory stimuli (Ozeki and Noma 1972).

The following two observations clearly indicate that there exists a separate class of specific receptor sites for TTX and STX: (1) exposure of the gustatory organ to known stimuli such as L-proline, betaine, AGPA, and TLCA had minimal effects on the responsiveness to TTX and (2) when TTX was used as an adapting stimulus, it failed to inhibit responses induced by all of the above-known gustatory stimulants except L-proline. However, the inability of L-proline and betaine to interfere with TTX receptors could partly be due to lesser potency of adapting stimuli relative to testing stimuli (e.g. 100% for 10^{-3} mol L-proline/L versus 200% and over for TTX at concentrations 2×10^{-6} mol/L and above). The fact that exposure to TTX at 5×10^{-6} mol/L significantly depresses L-proline responses, but not vice versa, suggests some pharmalogical effect on the gustatory transduction processes. Furthermore, evidence that TTX and bile salt stimulations are mutually independent and that amino acids and bile salts stimulate separate gustatory fiber types (S. Yamashita and T. J. Hara, unpubl. data) suggests different transduction mechanisms for bile salt stimulation.

The sensitivity to TTX was unaffected by adaptation to STX, and vice versa. These results, combined with the fact that they have distinct C–R relationships, provide strong evidence that the receptor(s) for TTX are different from those for STX in rainbow trout. The apparent differences in the C–R relationships between TTX and STX in Arctic char also support this contention. In this species, TTX receptors appear to have higher affinities (C–R curve shifted one order of magnitude to the left) than those for STX (shifted to the right). Further support to this is the recent experimental result that the gustatory sytem of a strain of Japanese rainbow trout lacks sensitivity to STX at all, while responding normally to TTX at concentrations comparable with those found in the present study (a specific anosmia; K. Yamamori, unpubl. data). Despite differences in the chemical structure, STX exerts essentially the same effect on nerve membranes as TTX, selectively blocking transient sodium channels (Narahashi 1974). One of the differences at the cellular level is the faster rate of action and recovery after STX than after TTX (Hille 1975). However, the finding that, unlike TTX, STX does not interact with the effects of chiriquitoxin, another TTX analog, has been interpreted as that the receptor for STX is different, but partially overlapping with that for TTX (Kao 1981). Further research is necessary to establish whether separate types of receptors for TTX and STX exist.

How can the TTX-containing animals such as puffers tolerate a concentration of TTX in their bodies, fatal enough for all otherwise normal animals? Studies have shown that puffers are more resistant to TTX than other fishes, e.g. 200–300 times that of goldfish (Saito et al. 1984). The nerve of the Atlantic puffer *Spheroides maculatus* is less sensitive to TTX than the frog nerve by a factor of 1000, but is highly sensitive to STX (Narahashi 1974). There appear to be at least two kinds of sodium channels, the common ones which are blocked by TTX and others which are not affected by TTX (Jacques et al. 1978).

It seems reasonable to assume that puffers might have evolved this alternate type of sodium channel as the predominant form in the presence of TTX. The inability of TTX to inhibit the gustatory responses elicited by other stimulants may also suggest that TTX-sensitive sodium channels are not primarily involved in the gustatory transduction process in rainbow trout.

It is generally accepted that puffers release TTX into the environment to prevent predators from attacking. Certain species secrete amounts as high as 10^{-5} mol TTX into the surrounding water, while being stimulated by repeated handling and electric shock (Saito et al. 1985; Kodama et al. 1985). Cultured, nontoxic puffers do not release any toxin; however, they become toxic and can release up to 2×10^{-7} mol TTX when fed with toxic puffer livers (Saito et al. 1985). Histological studies have shown that a unique exocrine gland exists in the skin of some puffers (Kodama et al. 1986). The gland is a large alveolar secretory and branched structure surrounded by a basement membrane, with an opening to the skin surface. Electrical stimulation causes contraction of microfilaments in the epithelial cells, resulting in secretion of the contents, in which a high concentration of TTX has been demonstrated (Kodama et al. 1986).

In summary, rainbow trout and Arctic char detect extremely low levels of the marine toxins TTX and STX via the specialized gustatory receptor(s). The results of cross-adaptation experiments, together with distinct C–R relationships, are relevant to the interpretation that these receptors are distinct from those which detect other known gustatory stimuli such as amino acids and bile salts. Furthermore, evidence is obtained to indicate that the receptor populations for TTX are distinct from those for STX. Apparent lack of toxic effect of TTX on bile salt stimulation suggests that different transduction processes between amino acids and bile salt receptors may exist. Evolution of the sensitive, specific receptor system for the toxins provides adaptative significance for predators. This is the first report on possible dual functions of marine toxins as semiochemicals providing adaptive significance both to producing and receiving organisms.

Acknowledgements

We thank Robert E. Evans, Freshwater Institute, for technical assistance and P. V. Cassidy for typing. K.Y. was supported by a travel grant from the School of Fisheries Sciences, Kitasato University.

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Effect of Diets Containing Herring Oil Oxidized to Different Degrees on Growth and Immunocompetence of Juvenile Coho Salmon (*Oncorhynchus kisutch*)

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Growth rate and efficiency of feed conversion were reduced by inclusion of oxidized herring oil in the diet, presumably as a result of reduced digestibility of the oxidized oil and suboptimal dietary concentrations of ω3 fatty acids. There was no evidence of any toxic factors in the oxidized oil. There was no significant difference in growth rate or the efficiency of feed conversion between juvenile coho fed diets supplemented with 30 and 1030 IU vitamin E/kg. Dietary treatment did not affect haematocrit values. Immunocompetence, judged by antibody titres in response to vibrio vaccination, was similar for all treatments. Disease resistance, assessed by the rate of mortality induced by exposure of nonvaccinated fish to challenge with *Vibrio anguillarum* or *V. ordali*, was likewise unaffected by the dietary treatments.

On a constaté que le taux de croissance et l'efficacité de la conversion des aliments baissent lorsque de l'huile de hareng oxydée entre dans l'alimentation, probablement parce qu'il s'agit d'une huile peu digestible et que la concentration d'acides gras ω3 alimentaires est inférieure à la valeur optimale. Rien n'indique que l'huile oxydée renferme des composés toxiques. On a étudié des saumons coho juvéniles dont l'alimentation était complétée par 30 et 1030 UI de vitamine E/kg : leur taux de croissance ne présentait pas de différence significative, ni non plus l'efficacité de la conversion des aliments. Le traitement alimentaire n'a pas modifié les valeurs de l'hématocrite. L'immunocompétence, évaluée d'après la production d'anticorps en réaction à un vaccin de vibrions, était la même, quel que soit le traitement. La résistance aux maladies, évaluée d'après le taux de mortalité causée par réaction provoquée à *Vibrio anguillarum* ou *V. ordali* chez des poissons non vaccinés, n'était pas modifiée non plus par le traitement alimentaire.

Received April 19, 1988
Accepted August 10, 1988
(J9703)

Reçu le 19 avril 1988
Accepté le 10 août 1988

Oxidized lipids in animal diets may have adverse nutritional effects for several reasons. Oxidation may reduce the digestibility of the lipids (March et al. 1965; Opstvedt 1973). The concentration of essential fatty acids in oxidized oils may be reduced because, being polyunsaturated, these fatty acids are especially prone to oxidation. Some of the oxidation products of lipids are themselves highly reactive and may destroy labile vitamins and reduce availability of some essential amino acids (El-Lakany and March 1974a, 1974b) and may diminish the activities of some enzymes (Tappel 1973). Oxidation products of lipids may be directly toxic to the animal if they are absorbed (Olcott and Dolev 1963; Kokatnur et al. 1966) although lipid peroxides may not be absorbed across the intestinal mucosa (Glavind and Tryding 1960). The presence

of oxidized lipid or of lipid that is undergoing autoxidation in a fish diet may accordingly induce a variety of effects. Cowey et al. (1984), for example, reported poor growth and efficiency of feed conversion in rainbow trout (*Salmo gairdneri*) fed diets containing mildly oxidized marine lipid without antioxidant protection, whereas Hung et al. (1980, 1981) found no significant adverse effect of oxidized dietary lipid in trout even though the concentrations of ω3 fatty acids were much reduced as a result of lipid oxidation.

Dietary vitamin E is one of the nutrients subject to oxidation in the presence of oxidized or autoxidizing lipid. Vitamin E has been demonstrated to be important for the immune response in various species of animals (Molenaar et al. 1980; Tendergy 1980). Beisel (1982), in a review of the effects of individual nutrients on immunity, concluded that intake of α-tocopherol in moderate excess of the recommended intake often increases the immune response in animals of a number of species. Blazer and Wolke (1984) reported that rainbow trout, fed a vitamin E deficient diet but showing no signs of vitamin E deficiency, nevertheless exhibited poor humoural and cellular immune responses. In a review of vitamin E and immune regulation, Corwin and Gordon (1982) suggested that recommended daily intakes of vitamin E may not be adequate for full immunocompetence.

The present experiment was conducted to examine the effects of diets containing herring oils oxidized to varying degrees on growth, efficiency of feed conversion, and immunocompetence in juvenile coho salmon (*Oncorhynchus kisutch*). The design of the experiment allowed for identification of possible effects of (1) loss of ω3 fatty acids, (2) increased dietary requirement for vitamin E in the presence of oxidized or autoxidizing lipids, (3) reduced availability of oxidized lipid, (4) lowered immune response, and (5) some unidentified growth suppressant(s).

Materials and Methods

Dietary Treatments

The composition of the basal diet fed in the experiment is shown in Table 1. The diet was semipurified and contained freeze-dried pollock muscle and freeze-dried euphausids as sources of protein. The diets were cold-pelleted (Higgs et al. 1979). Part of the dietary oil (8.73%) was reserved when the diets were mixed and was subsequently sprayed onto the pellets after their extrusion. Pellet size was initially 1.98 mm (5/64 in.) and was increased to 2.38 mm (3/32 in.) when the fish were about 8.5 g in weight. The pelleted diets were stored in plastic bags in the dark at 0–6°C throughout the experiment. Fatty acid analysis of the dietary lipid was conducted after 7.5 mo. Iodine values were determined on the dietary lipid after 8 mo. Vitamin E analyses of the diets were conducted after 10 and 22.5 wk. Vitamin C analyses were conducted after 1, 17, and 36 wk.

All except one of the experimental treatments involved diets supplemented with herring oil (HO) that had been treated in different ways. Samples of a commercial unstabilized HO (courtesy of British Columbia Packers, Ltd., Richmond, B.C.) were oxidized and/or stabilized as indicated below. The experimental treatments were as follows:

(1) Basal diet with the inclusion of untreated HO.

(2) Basal diet with the inclusion of HO that had been stabilized upon receipt with 500 ppm ethoxyquin (EQ).

(3) Basal diet with the inclusion of HO that had been heated with aeration to 35–50°C for ~15 d, followed by stabilization with 500 ppm EQ.

(4) Basal diet with the inclusion of HO that had been heated with aeration to 35–50°C for ~64 d, followed by stabilization with 500 ppm EQ.

(5) Basal diet with the inclusion of corn oil.

(6), (7), (8), and (9). Diets as in treatments 1, 2, 3, and 4, respectively, but with the further addition of 1000 IU vitamin E/kg, i.e. a total of 1030 IU/kg, since the basal diet was already supplemented with 30 IU/kg.

(10) Basal diet with the inclusion of a 1:4 mixture of unheated HO and HO that had been oxidized for 64 d, stabilized with 500 ppm EQ.

TABLE 1. Composition of the basal diet (as fed).

Ingredient (g/kg)		Mean analysis, dry weight basis (moisture 10.6%)	
Freeze-dried pollock muscle	425.34	Crude protein	48.6%
Freeze-dried euphausids	46.53	Lipid	16.5%
Dextrin	31.73	Ash	6.4%
Glucose	31.73	Calcium	9.82 g/kg
Ground cellulose	138.60	Phosphorus	8.58 g/kg
Vitamin supplement[a]	36.34	Magnesium	1.76 g/kg
Mineral supplement[b]	45.90	Zinc	95.8 ppm
Oil source	134.82	Copper	10.8 ppm
Carboxymethylcellulose	27.30	Iron	231.0 ppm
Ascorbic acid	1.82	Manganese	85.4 ppm
Choline chloride (60%)	4.55		
Ethoxyquin[c]	0.07		
Water	75.27		

[a]The vitamin supplement supplied the following levels of nutrients/kg dry diet: D-calcium pantothenate, 165.7 mg; pyridoxine·HCl, 36.5 mg; riboflavin, 60.0 mg; niacin, 300 mg; folic acid, 19.3 mg; thiamin mononitrate, 36.3 mg; biotin, 3.0 mg; cyanocobalamin, 60 mcg; menadione, 30.3 mg; cholecalciferol, 2400 IU; retinyl acetate, 10 000 IU; inositol, 400 mg; *dl*-α-tocopheryl acetate (diets 1, 2, 3, 4, 5, and 10, 30 IU; diets 6, 7, 8, and 9, 1000 IU).

[b]The mineral supplement supplied the following nutrients (mg/kg dry diet): calcium (as $CaHPO_4$, 3014; as $CaCO_3$, 5858), 8872; magnesium (as $MgSO_4 \cdot 7H_2O$), 663; manganese (as $MnSO_4 \cdot H_2O$), 74.6; zinc (as $ZnSO_4 \cdot 7H_2O$), 67; cobalt (as $CoCl_2 \cdot 6H_2O$), 4.00; copper (as $CuSO_4 \cdot 5H_2O$), 5.64; iron (as $FeSO_4 \cdot 7H_2O$), 191.1; fluorine (as NaF), 4.50; iodine (as KIO_3), 4.50; selenium (as Na_2SeO_3), 0.097; aluminum (as $AlCl_3 \cdot 6H_2O$), 34.1; phosphorus (as $CaHPO_4$), 2328.

[c]EQ was not added to diet 1 or 6.

(11) Pair-feeding of treatment 2 diet to feed intake of fish on treatment 1.

(12) Pair-feeding of treatment 2 diet to feed intake of fish on treatment 3.

(13) Pair-feeding of treatment 2 diet to feed intake of fish on treatment 4.

The diets contained 48.6% protein, 16.5% lipid, 6.4% ash on a dry weight basis, and 10.6% moisture by analysis.

Experimental Conditions

A population of 7-mo-old juvenile coho salmon from Capilano Hatchery (North Vancouver, B.C.) was selected for body weights between 3.6 and 6.4 g. The fish were distributed at random into 26 tanks, 112 fish/tank. The average weight of the fish at the beginning of the experiment was 5.1 g. The 13 dietary treatments were assigned at random to the tanks with duplicate tanks of fish on each treatment.

The fibreglass tanks each had a capacity of 140 L. Dechlorinated city water was provided to each tank at a flow rate of 1.8–2.0 L/min. Water temperature was not controlled and varied from a high of 13.0°C at the beginning of the experiment to a low of 4.5°C during the experiment. Lighting was constant at 15.7 h/d.

The fish were initially fed to near-satiation three times daily. Near-satiation was assumed when feed pellets fell to the bottom of the tank without being consumed. When water temperature decreased and feed consumption declined, the fish were fed twice daily. The daily feed consumption of the fish in each tank was recorded.

Body weights were determined at intervals of 3 or 6 wk, depending on the growth rate. At each weighing, 60 fish from each tank were anaesthetized with 2-phenoxyethanol (0.5 mL/L of water) and individually weighed. Mortality was recorded throughout the experiment. All the fish were weighed at 205 d when the growth study part of the experiment was completed.

After the final weighing, nine of the nonvaccinated fish (see below) from each tank were frozen whole at −18°C in plastic bags and stored for lipid analysis.

Tests for Immunocompetence

On day 169 of the experiment, 37 fish, selected at random from each tank, were vaccinated against vibriosis. The fish were immersed for 3 min in an aerated vaccine solution (Vibrogen, Connaught Laboratories, Willowdale, Ont.). The vaccine solution consisted of 1 L of Vibrogen, 9 L of 1% saline, and 1 mL of an antifoamant (Baker Antifoam B). The vaccinated fish were kept in tanks in another room to prevent cross-vaccination of the nonvaccinated fish. The nonvaccinated fish were handled similarly to the vaccinated fish at this time, except for the actual vaccination procedure, and were returned to their "home" tanks. Experimental conditions in the two tank rooms were similar. After 1 wk the vaccinated fish were anaesthetized and their adipose fins removed to identify the vaccinated fish. The vaccinated fish were then returned to the original tank room. It was assumed that there would be no residual active vaccine remaining at this time, since these bacteria are not viable in fresh water.

Thirty-six days after vaccination (day 205 of the experiment), blood samples were taken from nine nonvaccinated and 15 vaccinated fish from each tank of fish except for those on treatments 11, 12, and 13. The caudal peduncle of the anaesthetized fish was severed and blood samples from each fish taken into two microhaematocrit tubes. The tubes were centrifuged and the packed cell volumes measured. The tubes were then broken to separate the cells from the plasma. The tubes containing the plasma were sealed with plasticine and held in frozen storage pending measurement of antibody titres.

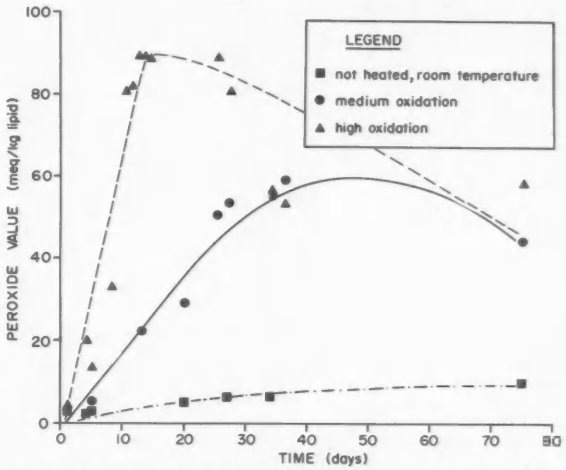

FIG. 1. Peroxide development in HO's kept at room temperature (not heated), heated for 15 d (medium oxidation), and heated for 64 d (high oxidation).

Antibody titres against *Vibrio anguillarum*, one of the antigens in the vaccine, were determined on the plasma samples by standard microtitration technique. Rabbit antiserum (Connaught Laboratories, Willowdale, Ont.), substituted for the fish plasma, provided the positive control for the tests. Plasma samples from approximately nine vaccinated and 15 nonvaccinated fish on treatments 2, 3, 4, 5, 7, 8, 9, and 12 were tested.

Some of the plasma samples (at least five from vaccinated fish and two from nonvaccinated fish per treatment) were tested for their ability to agglutinate or immobilize live *Vibrio ordalii* organisms. A sample of a culture of highly motile *V. ordalii* was mixed with an equal volume of test plasma on a hanging-drop slide and examined under oil immersion with a light microscope. The motility and agglutinating tendencies of the bacteria were compared subjectively with a positive standard (motile organisms plus commercial rabbit antiserum) and a negative control (motile organisms plus saline). Observations were begun 3 min after mixing and lasted several minutes. A sample of plasma from a coho salmon survivor of a vibrio challenge served as a control.

Forty-four days after vaccination the fish were challenged with live *V. anguillarum* to compare the susceptibility of the nonvaccinated fish on the different dietary treatments and the effectiveness of the vaccination for the fish on the various diets. The fish from each tank were immersed for 15 min in a saline (1%) suspension of *V. anguillarum* containing 10^9 viable bacteria/mL. New bacterial suspensions were prepared for the treatment of the fish in each tank. Water temperature at the time of vaccination was 8°C. No mortality occurred in response to the challenge, possibly because of the low water temperature that prevailed.

A second challenge was imposed 69 d subsequent to the vaccination. The organism employed was a stock culture of locally isolated *V. ordalii*. The challenge procedure was similar to that used previously except that fish from four tanks containing fish

TABLE 2. ω3 fatty acid concentrations and iodine values of dietary lipid extracted after 7.5 and 8 mo of storage, respectively, at 0–6°C.

Diet	Treatment	Iodine value of HO added to diet	ω3 fatty acids		
			% of dietary lipid	% of dry diet	Iodine value of dietary lipid
1	Untreated HO	111	7.69	1.29	114
2	Stabilized HO	113	12.31	2.03	112
3	HO, heated 15 d stabilized	102	10.71	1.81	98
4	HO, heated 64 d stabilized	81	5.79	0.94	90
5	Corn oil		3.03	0.52	114
6	Diet 1 + vitamin E		10.08	1.66	109
7	Diet 2 + vitamin E		12.02	1.99	107
8	Diet 3 + vitamin E		10.40	1.68	109
9	Diet 4 + vitamin E		5.53	0.89	90
10	1:4 mixture of stabilized HO + HO heated 64 d, stabilized		7.23	1.20	97

TABLE 3. Vitamin E concentrations in diets sampled at 10 and 22.5 wk after preparation. All diets were stored at 0–6°C throughout the study. Dietary treatments were imposed 5 wk after preparation of the diets.

Diet	Treatment	Vitamin E concentration (IU/kg, dry weight basis)	
		10 wk	22.5 wk
1	Untreated HO	97	20
2	Stabilized HO	155	39
3	HO, heated 15 d stabilized	81	3
4	HO, heated 64 d stabilized	29	3
5	Corn oil	78	21
6	Diet 1 + vitamin E	833	368
7	Diet 2 + vitamin E	770	679
8	Diet 3 + vitamin E	908	608
9	Diet 4 + vitamin E	754	568
10	1:4 mixture stabilized HO + HO heated 64 d, stabilized	37	32

[a]Analyses by B. Dosanjh at a commercial analytical laboratory using the method of Soderhjelm and Andersson (1978). NRC (1981) recommended vitamin E requirement for salmonids, 30 IU/kg dry diet.

TABLE 4. Vitamin C concentrations[a] in diets sampled at different times after preparation. All diets were stored at 0–6°C throughout the study. Dietary treatments were imposed 5 wk after preparation of the diets.

Diet	Treatment	Vitamin C concentration (mg/kg, dry weight basis)		
		1 wk	17 wk	36 wk
1	Untreated HO	1480	580	310
2	Stabilized HO	1570	520	250
3	HO, heated 15 d stabilized	1480	520	270
4	HO, heated 64 d stabilized	1490	750	340
5	Corn oil	1520	850	440
6	Diet 1 + vitamin E	1520	820	270
7	Diet 2 + vitamin E	1480	860	390
8	Diet 3 + vitamin E	1520	630	270
9	Diet 4 + vitamin E	1400	550	370
10	1:4 mixture stabilized HO + HO heated 64 d stabilized	1540	630	390

[a]Analyses by B. Dosanjh at a commercial laboratory using the indophenol method of AOAC (1980). NRC (1981) recommended vitamin C requirement for salmonids, 100 mg/kg dry diet.

on treatments 5, 9, 11, and 12 were immersed in the bacterial suspension for 25 min and all other fish were immersed for 14 min. It was considered from observation of the fish that the longer immersion period stressed the fish too severely. Mortality rates were recorded for each tank of fish.

Chemical Analyses

Proximate analyses of the test diets were conducted according to the AOAC (1984) procedures except for the determination of lipid concentration which was accomplished by the method of Bligh and Dyer (1959). The mineral compositions of the test diets were determined using an Inductively Coupled Argon Plasma Emission Spectrometer (ICAP-AES), Jarrell Ash model 975 Plasma Atomcomp, as previously described by Higgs et al. (1982). Vitamin E and vitamin C analyses of the diets were carried out according to the methods of Soderhjelm and Andersson (1978) and AOAC (1984), respectively.

The various herring oils used in the diets were analyzed for peroxide value during the course of the oxidative treatment (AOCS 1973). Wij's iodine values (AOCS 1980) were determined on the oils at the time that the diets were prepared and on the lipids extracted from the diets after storage.

Samples of the lipid extracted from the diets were esterified according to the base-catalyzed methyl esterification procedure of Christie (1982). Fatty acid composition was measured using a Varian 3700 gas–liquid chromatograph equipped with a Supelco SP-2330 capillary column, 30 m × 0.25 mm inside diameter (Supelco Canada Ltd., Oakville, Ont.). The temperature program was set at 180°C for 10 min, and increased 5°/min to 195°C. Flow rate was 40 mL/min. Peaks were identified relative to fatty acid methyl ester standards, primarily PUFA-1 (Supelco Canada Ltd.).

TABLE 5. Mean body weight gains (BWG) and values for efficiency of feed conversion (BWG/FI), 0–205 d.

Diet	Treatment	BWG (g)[a]			BWG/FI (g/g)[b]		
			Added vitamin E	Pair-fed diet 2		Added vitamin E	Pair-fed diet 2
1	Untreated HO	14.67	(6) 13.02	(11) 15.62	(1) 0.868	(6) 0.826	(11) 0.933
2	HO, ethoxyquin (EQ) added	16.03	(7) 15.78	—	(2) 0.916	(7) 0.905	—
3	HO, heated 15 d EQ added	13.31	(8) 14.55	(12) 14.40	(3) 0.874	(8) 0.846	(12) 0.941
4	HO, heated 64 d EQ added	13.51	(9) 15.15	(13) 14.56	(4) 0.764	(9) 0.774	(13) 0.888
5	Corn oil	13.54			(5) 0.795		
10	Untreated HO:HO heated 64 d, 1:4, EQ added	14.99			(10) 0.805		

[a]Body weight gains not significantly different on the basis of an overall analysis of variance. SEM of all means = 1.080.

[b]Analysis of variance of values for efficiency of feed conversion by fish fed diets to near satiation. Main effects: state of oxidation, $p < 0.001$; 30 versus 1000 IU vitamin E/kg diet, not significant; interaction, not significant. The following mean values with different superscripts are significantly different ($p < 0.05$): untreated HO, 0.846[y]; HO, EQ added, 0.910[x]; HO, heated 15 d, EQ added, 0.860[y]; HO, heated 64 d, EQ added, 0.769[z]; SEM = 0.0142.

TABLE 6. Mortality of nonvaccinated fish subsequent to challenge with *V. ordalli*.

Diet	Treatment	Total no. of fish	% mortality
1	Untreated HO	149	14.8
2	Stabilized HO	444[a]	13.1
3	HO, heated 15 d, stabilized	151	21.2
4	HO, heated 64 d, stabilized	150	10.0
5	Corn oil	74[b]	13.5
6	Diet 1 + vitamin E	73[b]	4.1
7	Diet 2 + vitamin E	57[b]	7.0
8	Diet 3 + vitamin E	150	15.3
9	Diet 4 + vitamin E	150	17.3
10	1:4 mixture stabilized, HO + HO heated 64 d, stabilized	149	9.4

[a]Includes pair-fed fish.

[b]One tank of fish only; fish from the replicate tanks were accidently lost from the experiment.

The nine fish sampled from each tank were pooled for the determination of dry weight and lipid analysis. The fish were thawed and homogenized in a Waring blendor. Triplicate Aliquots of the homogenate were taken for determination of dry matter and lipid concentrations. Lipid concentrations in the fish were determined by the method of Bligh and Dyer (1959).

Statistical Analyses

Values for body weight gains, efficiency of feed conversion, and packed cell volume were subjected to analyses of variance (Snedecor and Cochran 1980). Treatment mean differences were considered significant at the 5% overall level of probability. Analysis was done on all treatments, as well as factorially on mean body weights for treatments 2, 3, 4, 7, 8, and 9. Mean body weights for all weighings were included in a single factorial analysis using a third-order forward stepwise regression (Snedecor and Cochran 1980).

Results

Peroxide Values of Dietary HO's

The changes in the peroxide values of the oxidized HO's during the heating period and the values for the HO that was stabilized initially and stored at room temperature are shown in Fig. 1. The unheated HO (the oil not stabilized with EQ, as well as the oil that was stabilized initially with EQ) had developed a peroxide value of only 10 meq/kg oil at the time that the oils were mixed into the diets. The oil that was heated for 15 d reached a maximum peroxide value of 60 and had declined to 44 at the time of diet preparation. The highly oxidized oil reached a peroxide value of 90 and had declined to 58 at the time of diet preparation.

Iodine Values of HO's and Total Dietary Lipid

Iodine values of the oils that were not heated were similar regardless of whether or not the oil was stabilized with EQ (Table 2). Rigorous heating and aeration reduced the iodine value to 81 and this lower iodine value was reflected in the iodine value of the lipid extracted from the diets to which it was added. The iodine value of the oil extracted from the diets containing the mixture of unheated oil and oil heated for 64 d was intermediate between the iodine values for the diets containing the respective oils individually.

Fatty Acid Composition of Dietary Lipid

Although EQ stabilization did not measurably affect the iodine value of the dietary lipid, the concentration of ω3 fatty acids was 12.3% of the lipid extracted from the stabilized diet

2 compared with 7.69% of the lipid from the unstabilized diet 1. The concentration of ω3 fatty acids in lipid extracted from the diets containing the severely oxidized HO was only between 5 and 6%. The essential fatty acid concentrations in these diets were accordingly below the recommended 1% for coho (Yu and Sinnhuber 1979; Takeuchi and Watanabe 1982) as was the case with diet 5 which contained corn oil instead of HO (Table 2).

Comparison of the iodine values of lipid extracted from the diets after 8 mo of storage with the iodine values and fatty acid analyses of the supplementary oils indicates that little lipid oxidation had occurred during storage of the diets.

Dietary Vitamin E and C

The vitamin E concentration in diet 2 at 10 wk suggested that the fresh stabilized HO contributed considerable vitamin E to the diet (Table 3). When the HO added to the diets had not been stabilized with EQ, or had been deliberately oxidized prior to stabilization, there was loss of vitamin E which was reflected in the lower concentrations of the vitamin in diets 1, 3, 4, and 10. The concentrations of vitamin E in diets 1, 3, 4, 5, and 10 declined to below the NRC (1981) recommendation of 30 IU/kg diet by 22.5 wk. The analytical technique employed was not sufficiently sensitive to distinguish among values at the lower range of concentrations.

The temporal changes in the dietary concentrations of vitamin C (Table 4) revealed a pattern of decline that was similar for all diets regardless of the extent of dietary oxidation or of dietary concentration of vitamin E. The vitamin C concentrations of the diets after 36 wk of storage were 2.5 to 4.4 times higher than the NRC (1981) recommendation of 100 mg/kg diet.

Body Weight Gains and Feed Efficiency

The mean values for body weight gains during the 29-wk experimental period and for feed efficiency, expressed as body weight gain per feed intake, are given in Table 5. The analyses of variance for body weight gains using only the initial and final body weights revealed no significant treatment effects. However, when the data from all the weighing times for treatments 2, 3, 4, 7, 8, and 9 were analyzed using stepwise regression in a 2 × 3 factorial fashion, there was a significant cubic interaction of time with the degree of HO oxidation ($p < 0.05$) but no significant effect of added vitamin E. Fish fed diets containing the untreated HO (EQ stabilized) consistently grew at the fastest rate. Fish fed the diets containing the oil that had been heated and aerated for 15 d had the poorest rate of growth.

Efficiency of feed conversion was significantly ($p < 0.01$) reduced by oxidation of the supplementary HO but vitamin E had no significant effect. Fish fed diet 2, in the amounts corresponding to the feed consumption of the fish fed diets 1, 3, and 4, respectively, grew faster than those fed the latter diets and they also exhibited more efficient feed conversion. Analysis of variance of the values for efficiency of feed conversion with treatments 1–4 and 6–9 showed significant differences associated with the oxidative state of the HO. Dietary inclusion of the oil that had been heated for 64 d resulted in the poorest conversion, whereas the oil that was unheated but stabilized gave the best efficiency. Addition of the high levels of vitamin E to the diets did not significantly affect efficiency of feed conversion.

Hematocrit Values

The mean hematocrit values for the nonvaccinated fish on the various treatments varied from 44.8 to 51.4%. There was no significant dietary effect. The mean hematocrit value for the vaccinated fish was 45.3% which was significantly lower ($p < 0.01$) than the mean value of 47.2% for the nonvaccinated fish.

Immunocompetence

Measurements of antibody titres and agglutination characteristics showed no significant differences among the fish on the different dietary treatments. Mortality data for the nonvaccinated fish following their exposure to live *V. ordalii* are shown in Table 6. Nearly all of the fish that died were positive for the organism. Vaccination consistently gave protection against the challenge. Only two of the vaccinated fish from all dietary treatments died.

Discussion

Growth and Efficiency of Feed Conversion

The experimental treatments in this study consisted of a core set of dietary treatments, by means of which the effects of dietary lipid oxidization and vitamin E concentration on growth, feed efficiency, and immunocompetence of coho were examined, and several auxiliary treatments were included to investigate specific associated hypotheses. The growth data from the core treatments, i.e. Nos. 2, 3, 4, 7, 8, and 9, when analyzed in a 2 × 3 factorial fashion, demonstrated a significant cubic interaction of time with lipid oxidation ($p < 0.05$), but no effect of vitamin E concentration. The cubic relationship of mean body weight to time is attributable to the change in water temperature during the period over which growth was measured. On the basis of the findings of Cowey et al. (1984), regarding the effects of oxidized oil on rainbow trout, such effects would be expected to be more apparent at low than at high (e.g. 15°C) water temperature. Fish fed the diets containing oil that had undergone the least oxidation consistently grew most rapidly; those fed diets containing moderately oxidized oil showed the slowest growth rate. Feed consumption by the latter group was, however, less than that of the fish fed the highly oxidized oil.

Efficiency of feed conversion (body weight gain/feed intake) was influenced significantly ($p < 0.01$) by experimental treatment (Table 5). A two-way factorial analysis of variance of the feed conversion data from the core set of treatments demonstrated that the oxidation state of the HO in the diet, but not the dietary concentrations of vitamin E, affected feed efficiency. The faster rate of growth but the poorer feed efficiency observed for the fish fed the highly oxidized oil can be explained by a reduction in the digestibility of the highly oxidized oil, and consequently in the available energy concentration in the diet containing this oil (March et al. 1965; Opstvedt 1973). Therefore, to satisfy energy requirement, the fish fed the diet supplemented with the highly oxidized oil had a higher daily intake of other nutrients which can account for their faster rate of growth if the energy to nutrient ratio was above the optimal in the other diets.

The effects of the oxidation state of the HO on the efficiency with which the diets were utilized were not significantly modified by the addition of vitamin E to the diets. Even when the HO was not subjected to heating, feed efficiency was improved when the oil was stabilized upon its receipt and the diet was further stabilized with EQ to a total of 137 ppm. It is also notable that when diet 2 (unheated HO stabilized with EQ) was pair-fed, in treatments 11, 12, and 13, to the feed

consumption by fish fed diets 1, 3, and 4, respectively, feed efficiency was improved in every instance.

The improved efficiency of feed conversion that resulted when diet 2 was pair-fed to the consumption of fish fed the other HO-containing diets and the higher intake of ω3 fatty acids from diet 2 suggest that a deficiency of essential fatty acids, as well as lowered oil digestibility, had a role in limiting feed efficiency of fish fed the diets containing oxidized HO. The relatively poor feed conversion with the corn oil diet, which was likewise low in essential fatty acids, but which can be assumed to be readily digestible, supports this conclusion.

There was no evidence that vitamin E concentration in any of the test diets was inadequate for growth or for efficiency of feed conversion. Cowey et al. (1983) found that rainbow trout showed no growth response to supplementary vitamin E even when the diet did not contain sufficient vitamin E to maintain normal erythrocyte stability. McCay (1985), in a review of the interactions of vitamin E with free radicals and ascorbate, concluded that if there is a reaction in vivo between ascorbate and tocopherol radicals (similar to that occurring in in vitro systems), it may be difficult to produce a vitamin E deficiency in the presence of ascorbic acid. McCay (1985) discussed several mechanisms whereby ascorbic acid may interact with tocopherol to protect the animal against the consequences of lipid oxidation. Throughout the present experiment the dietary concentrations of vitamin C remained well above the recommended levels. Smith (1979) found that rainbow trout fed a rancid diet supplemented with vitamin E and ascorbic acid performed as well as control fish fed a nonrancid diet. The deficiency signs that developed in the fish fed the rancid diet without vitamin E and C were typical of vitamin E deficiency.

Immunocompetence

An objective of the experiment was the examination of the effect of oxidized HO on the immune response and resistance to vibriosis at two dietary concentrations of vitamin E. The basal concentration of vitamin E added to the diets, 30 IU/kg, is the requirement as stated by NRC (1981). It could not be assumed that the HO would be a consistent source of the vitamin, given the oxidizing treatments to which some of the HO's were subjected. It is apparent, however, that the fish did not suffer a vitamin E deficiency on any of the diets. Diet did not significantly affect the rate of mortality of unvaccinated fish infected with *V. ordalii*. The data do not, however, preclude the possibility that significant dietary effects might have been identified had more fish been employed or if the fish had been reared at a higher water temperature. The immune response of fish vaccinated against vibriosis was similar for fish on all dietary treatments as judged by either agglutination titre or by protection against challenge by *V. ordalii*. It is important to note that, prior to vibrio challenge, only four fish had died from the entire population (other than a few fish that were lost accidentally).

Conclusions Regarding Nutritional Properties of Oxidized HO

The results of this experiment indicate that, although oxidation reduced the essential fatty acid concentration in the oil and likely reduced the digestibility of the oil so that the available energy concentration in a diet containing highly oxidized oil was also reduced, the oil itself was not toxic. The accelerated oxidation promoted by heating of the oil during aeration would have enhanced the rate of peroxide destruction (Markley 1947), hence the failure of the heated oils to develop high peroxide values. The oxidation products, produced at the temperatures applied, may have been different from those forming at lower temperatures (March et al. 1961). The dietary conditions provided in this experiment provided considerable protection to the fish against secondary effects of oxidized oils mediated via induction of nutrient deficiency. The findings with coho salmon are thus similar to those reported previously for chicks (March et al. 1962). That study demonstrated that a growth-depressing effect of oxidized HO fed to chicks was overcome when the diet was adequately fortified with vitamins. In the present study, supplementation of the diets with appropriate levels of minerals, e.g. selenium, copper, zinc, and manganese, may have also exerted a protective effect via mechanisms considered by Bell and Cowey (1985).

Acknowledgements

This paper is based on the M.Sc. thesis of I.F. The study was supported by funding to I.F. from the Department of Fisheries and Oceans.

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Effects of Winter Floods on Fishes in the Sierra Nevada

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Winter floods in the Sierra Nevada mountains kill age 0 class brook trout (*Salvelinus fontinalis*) and Paiute sculpin (*Cottus beldingi*) because bed-material transport increases greatly when high flows are constrained by snow banks. In February 1982, dead Paiute sculpin were collected while sampling bedload during a rain-on-snow flood. Population estimates by electrofishing at nine permanent stations the following summer showed that density (3586·ha^{-1}) and biomass (12.9 kg·ha^{-1}) of Paiute sculpin were lower than the respective means (12 017·ha^{-1} and 40.3 kg·ha^{-1}) obtained during previous studies from 1952 to 1961. These estimates were also below those obtained in 1956, after the largest winter flood from 1952 to 1961. Brook trout fry were also less abundant in 1982 than previously reported. Maximum flow depth, rather than discharge, were the likely cause of fish mortality. Winter floods are severe because accumulated snowpack increases the effective height of the streambank and confines most or all of a rain-on-snow flood within the channel. Shear forces on the bed increase and as a result bed-material transport increases rapidly. These conditions directly kill many benthic-living fishes such as the Paiute sculpin or buried eggs of fall-spawning fishes such as the brook trout by mechanical grinding or crushing. The impact of snow-constrained floods was not uniform along Sagehen Creek due to patchiness in types of riparian canopy. The relationship among canopy cover, snow accumulation, and winter floods points to one more critical role that buffer strips may play in ameliorating effects of timber harvesting.

Les crues hivernales dans la Sierra Nevada détruisent les ombles de fontaine (*Salvelinus fontinalis*) et les chabots (*Cottus beldingi*) de la classe d'âge 0 parce que le transport de matériaux de fond s'intensifie beaucoup lorsque s'élève le débit des eaux, celles-ci étant davantage retenues entre les berges à cause de la neige qui s'y accumule. En février 1982, on a recueilli des chabots morts lors d'une opération d'échantillonnage des matériaux de fond durant une crue nivo-pluviale. L'été suivant, d'après les estimations de population faites par électropêche dans neuf stations permanentes, la densité (3 586·ha^{-1}) et la biomasse (12,9 kg·ha^{-1}) de chabots étaient inférieures à la moyenne (12 017·ha^{-1} et 40,3 kg·ha^{-1} respectivement) déterminée lors d'études réalisées de 1952 à 1961. Les valeurs estimées étaient également inférieures à celles qu'on avait déterminées en 1956 après la crue hivernale la plus importante à être observée de 1952 à 1961. Le frai d'ombles de fontaine était aussi moins abondant en 1982 que les autres années. On pense que c'est la profondeur maximale d'écoulement, non le débit, qui est la cause probable de la mortalité des poissons. Les crues hivernales sont lourdes de conséquences parce qu'avec l'accumulation de la neige, les berges sont plus hautes, de sorte qu'elles retiennent dans le lit la plus grande partie sinon toute l'eau nivo-pluviale. Comme les forces de cisaillement qui s'exercent sur le lit augmentent, le transport des matériaux de fond s'intensifie rapidement. Ces conditions mènent directement à la destruction par broyage mécanique d'un bon nombre de poissons de fond, comme le chabot, ainsi que des oeufs enfouis par les poissons qui fraient l'automne comme c'est le cas de l'omble de fontaine. L'effet des crues hivernales n'est pas le même partout dans le cours de la Sagehen, car la végétation riparienne n'est pas uniforme. La relation observée entre la végétation, l'accumulation de neige et les crues hivernales porte à penser que les bandes tampons rempliraient de ce point de vue une fonction essentielle dans l'atténuation des conséquences des coupes.

Received August 12, 1987
Accepted August 10, 1988
(J9384)

Reçu le 12 août 1987
Accepté le 10 août 1988

Stream flooding has long been recognized as a major influence on biota and physical structure of the channel (Tarzwell 1937; Needham 1938). The consequences range from the deleterious effects of destruction of fish year-classes and reduction of benthic invertebrate standing crops (Needham and Jones 1959; McFadden and Cooper 1962; Elwood and Waters 1969) to the beneficial, such as presumed flushing out of fine sediment deposits (Allen 1951). In a review of long-term studies on salmonid populations, Hall and Knight (1981) concluded that floods were one of the most common sources of natural variation in fish density and biomass.

In Sagehen Creek, California, a major winter flood caused by rainfall on an existing snowpack occurred during February

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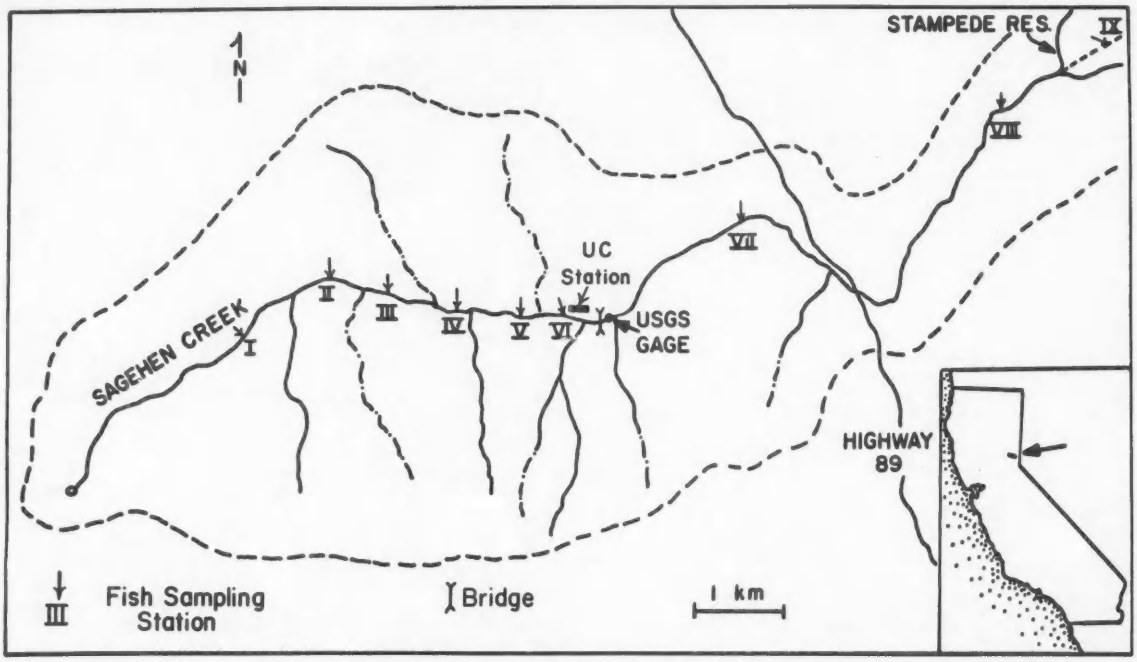

FIG. 1. Sagehen Creek showing the location of permanent fish sampling stations, the USGS streamflow gage, footbridge for collecting bedload samples, and the University of California (UC) Sagehen Creek Field Station.

1982. While sampling bedload, we collected, but did not save, many dead Paiute sculpin (*Cottus beldingi*) of all size-classes along with coarse sediment. To our knowledge, these sculpin were rare physical evidence of direct mortality from a flood. Prior studies at Sagehen Creek showed that heavy winter floods reduced the density of young-of-the-year (YOY) brook trout (*Salvelinus fontinalis*) the following summer (Seegrist and Gard 1972). The reduction was most pronounced in the summer 1956 survey after a December 1955 flood of 14 $m^3 \cdot s^{-1}$. Although winter floods usually exceed the discharge of spring floods, the difference is not large. Hence, the question arises why winter floods seem to exert such a great effect on some fish populations in Sagehen Creek. The objectives of our study were to look for fish in subsequent bedload samples taken during periods of flood discharge, to examine concomitant hydrologic conditions, and to determine if densities of YOY brook trout and Paiute sculpin after the February 1982 flood were lower than expected. We think that the conclusions from this analysis have implications for many streams, especially those subject to rain-on-snow events typical of the Sierra Nevada, the Cascades, and parts of the Rocky Mountains (Smith 1947; Harr 1981).

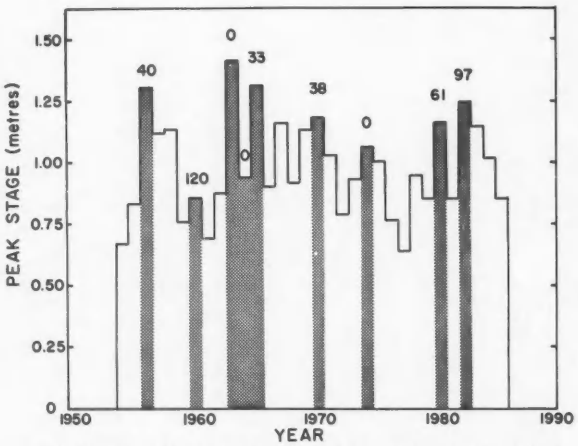

FIG. 2. Annual peak stage of Sagehen Creek at the gage since 1953. Winter peaks are shaded and the existing snowpack (cm) for the date of the peak is shown above each bar. The greatest peaks are in winter usually when snow raises the effective channel height.

Methods

Study Area

Descriptions of the Sagehen Creek area and permanent fish sampling stations have been given previously (Gard and Seegrist 1972; Erman 1986). The Sagehen Creek basin is located 16 km north of Truckee, California, and drains the east slope of the Sierra Nevada Mountains (Fig. 1). The basin has been subject to low-intensity grazing, logging, other land uses, and wild fire since the late 1800's. Land use since the early 1950's has changed little up to the present (Erman 1986). Channel stability and concentrations of suspended sediment are indicative of a stable watershed (Simpson 1974; Andrews and Erman 1986; Erman 1986). The median diameter of the bed surface material is 58 mm compared with 27 mm for the subsurface material (Andrews and Erman 1986).

A U.S. Geological Survey gage on Sagehen Creek (USGS No. 10343500) has recorded the water surface elevation (stage)

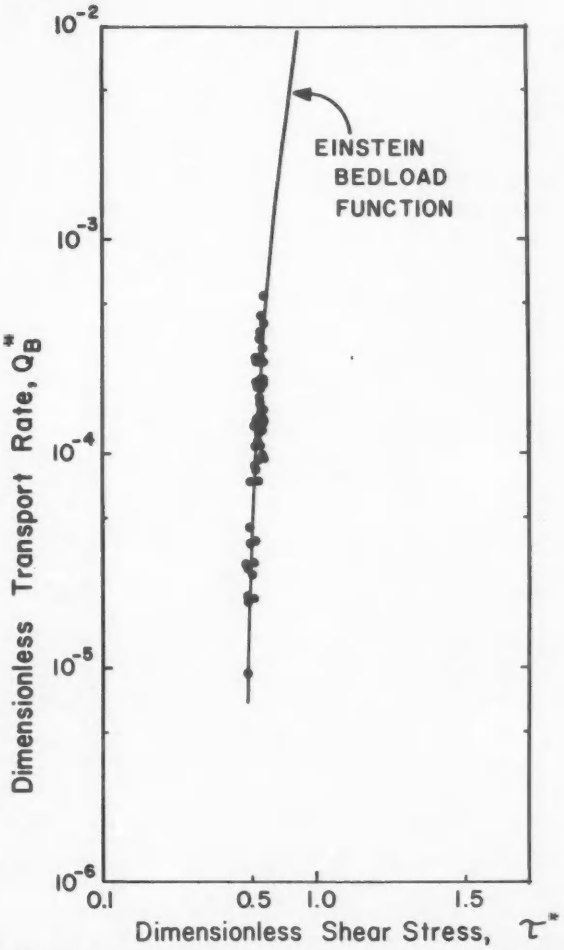

FIG. 3. Dimensionless transport rate of bed material in Sagehen Creek in relation to dimensionless shear stress. The 58 daily totals from 1982, 1983, and 1984 are in excellent agreement with the curve drawn from a function developed by Einstein (1950) and later modified by Parker (1979).

continuously since October 1953. Water discharge measurements are made several times a year to establish the relationship between river stage and discharge. Contributing area to the gaging station is approximately 27.2 km^2. Elevation of the basin ranges from 2670 m, along the crest, to 1926 m at the gaging station.

Mean annual precipitation recorded at a U.S. Weather Service station, located at the Sagehen Creek Field Station (Fig. 1), was 940 mm from 1947 to 1985. Winter snowfall accounts for 75% of the total precipitation. Typically, a snowpack begins to accumulate in mid-November and persists until late April or May. Snow depth is measured daily at the field station.

Flood discharges in Sagehen Creek occur as a result of winter rainfall, usually on an existing snowpack, and spring snowmelt. Winter floods last only 1–4 d and occur between November and March. In contrast, spring snowmelt floods are sustained, lasting several weeks, and usually occur from mid-April to mid-June.

Nine species of fish are present from the headwaters to Stampede Reservoir 21 km downstream. Brook trout, Paiute sculpin, brown trout (*Salmo trutta*), and rainbow trout (*Salmo gairdneri*) regularly inhabit the upper two thirds of the creek (above Highway 89). The lower sections include those species plus Tahoe sucker (*Catostomus tahoensis*), mountain sucker (*Catostomus platyrhynchus*), speckled dace (*Rhinichthys osculus*), Lahontan redside (*Richardsonius egregius*), and mountain whitefish (*Prosopium williamsoni*). Nine permanent fish sampling stations are spaced from the headwaters to the reservoir (Fig. 1).

Hydrology and Sediment Transport

To investigate the hydraulic and sediment movement characteristics of Sagehen Creek, we established a study reach about 200 m upstream from the streamflow gage. Bed material was sampled daily at the footbridge (Fig. 1) during the spring snowmelt floods of 1982, 1983, and 1984. A handheld Helley–Smith bedload sampler with a 150-mm square orifice was used to collect bed material (Helley and Smith 1971; Emmett 1980; Andrews 1981). Because of the large variability commonly observed in the bedload transport rate (Ehrenberger 1931), three traverses of the footbridge cross-section were made each day. During a traverse, we sampled each of four locations, spaced 0.61 m apart, for 4 min. The samples were dried, weighed, and sieved separately. The mean transport rate and size distribution of bed-material on a given day were determined by averaging twelve 4-min bedload samples.

Fish Populations

To estimate fish populations after the February flood, we sampled fish on August 28–30, 1982, at nine permanent study sections using a backpack electroshocker (Smith-Root type 5 and 11a). Fish were removed in two, or usually three, passes through a section blocked with seines (Erman 1986). Population estimates followed the method of Carle and Strub (1978). Captured individuals were weighed to the nearest gram (Pesola scale) and measured in total length to the nearest mm. Brook trout YOY were defined as fish less than 100 mm in total length as had been established earlier (Seegrist and Gard 1972). The earlier studies (1956–61) used a method of diversion-and-drain to census fish annually in late August (Gard and Seegrist 1972; Gard and Flittner 1974). The two methods give comparable results (Erman 1986).

Results

Flood Hydrology

Annual maximum flood stages (for water year, October 1 to September 30) at the Sagehen Creek gage are shown in Fig. 2. Between 1953 and 1985, 23 of the 32 annual maximum stages were the result of spring floods. The six highest flood stages occurred during winter floods.

Flood stage rather than discharge is shown in Fig. 2 because the actual discharge of the rain-on-snow floods is uncertain. In addition, water depth (stage) is the primary variable affecting shear stress on the bottom (Appendix). The stage–discharge relationship for the Sagehen Creek gage is determined when the streambanks and floodplain are free of snow. Consequently, the measured floodflows were not confined to the actual channel. In the vicinity of the gage, the bankfull stage is 0.870 m which

corresponds to a discharge of about 2.0 $m^3 \cdot s^{-1}$. At stages greater than 1.036 m, much of the flood discharge flows in the overbank areas. For example, the full discharge of Sagehen Creek on June 4, 1983, was 4.81 $m^3 \cdot s^{-1}$ at a stage of 1.052 m; however, 44% was flowing outside the channel.

During winter, the accumulated snowpack increases the effective height of the streambank and confines most or all of a rain-on-snow flood within the channel. The snowpack depth recorded at the time of each rain-on-snow flood is shown in Fig. 2. For several of the winter floods, the depth of accumulated snow was sufficient to greatly reduce or eliminate overbank flow. Consequently, a rain-on-snow flood will have a higher stage than a spring snowmelt flood with the same discharge. In particular, there were large accumulations of snow during the winter floods in 1956 (40 cm) and 1982 (97 cm).

Not all of the winter floods, however, have been affected by an existing snowpack. The maximum flood stage of record, February 1, 1963, was caused by several days of heavy rainfall when the lower part of the Sagehen Creek basin was essentially free of snow. In such instances, both the stage and discharge are high.

Bed-Material Transport

During the spring snowmelt floods, we measured bed-material transport rates on 58 occasions at discharges between 0.99 and 3.12 $m^3 \cdot s^{-1}$. Dimensionless bed-material transport rates, Q_B^*, are plotted in Fig. 3 as a function of the dimensionless shear stress, τ^*. The 58 daily totals from 1982, 1983, and 1984 are in excellent agreement with the curve drawn from a function developed by Einstein (1950) and later modified by Parker (1979). Derivation of the hydraulic parameters for shear stress and sediment transport are given in the Appendix.

During the period of record, 1953–85, a discharge of 1.00 $m^3 \cdot s^{-1}$ was equalled or exceeded 10% of the time or 36 $d \cdot yr^{-1}$ on average. Even though the discharges at which the bed-material transport rate was sampled in 1982–84 are among the highest ever recorded on Sagehen Creek, the flow conditions only slightly exceeded those required to initiate particle motion ($\tau_c^* = 0.043$, see Appendix). The observed range of τ^* was from 0.048 to 0.058. It is quite unlikely that values of τ^* ever exceeded 0.058 by more than a few percent in Sagehen Creek during the spring snowmelt flood. Any increase in flood discharge spreads across the floodplain and does not increase channel flow depth or bed shear stress appreciably.

The estimated mean annual load of bed material in Sagehen Creek for the period of record is 21 000 $kg \cdot yr^{-1}$. Nevertheless, measured bed-material transport rates are very small. A value of Q_B^* equal to 1×10^{-5} is equivalent to about 1 $g \cdot m$ channel $width^{-1} \cdot s^{-1}$, given the distribution of bed-material particle sizes. The maximum observed transport rate, 6.4×10^{-4}, is equivalent to 0.059 $kg \cdot m^{-1} \cdot s^{-1}$ (or the movement of one particle of median diameter, 58 mm, every 30 s for every metre of channel width). At the relatively small bed-material transport rates measured during spring floods, only a few bed particles are moving at any instant and the majority of particles larger than the median do not move at all in an average spring flood (Andrews and Erman 1986). By contrast, in the 1982 snowmelt flood (somewhat greater than average flow), 60% of median diameter particles and 8% of particles in the coarsest 10th percentile fraction were moving.

The steep relation between Q_B^* and τ^* means that a slight increase in τ^* greatly increases the movement of bed material. During the 1982, 1983, and 1984 spring floods, bed-material transport rates were sampled at stages as high as 1.103 m. Transport rates at such stages are small and have had little impact on fish populations. Winter floods of only slightly greater stages, 1.25–1.30 m, however, have had a significant impact.

Given the close fit of data shown in Fig. 3 to Parker's curve, we used his equation (Appendix) to estimate transport rates that occurred at the crest of all winter floods. At a stage of 1.25 m, the bed-material transport rate is about 4.4×10^{-3} or 10 times the estimated transport rate at a stage of 1.10 m. The estimated bed-material transport rate at the maximum stage of record, February 1, 1963, is approximately 2.0×10^{-2} or 45 times the estimated transport rate at a stage of 1.10 m. The rapid increase in bed-material transport rate is due primarily to an increase in the number of particles entrained by flow at any instant. At

TABLE 1. Density and biomass (± 90% CI) of Paiute sculpin at permanent stations in Sagehen Creek. Data from 1952 to 1961 (Gard and Flittner 1974 and original data) are from complete census of sections. Data from 1982, following a winter flood, are estimates and their 90% confidence intervals (Erman 1986). Values in 1956 were the lowest from 1952 to 1961 after a winter flood. No Paiute sculpin have been collected at section I. Confidence intervals could not be computed for density in sections II and III in 1982.

	Density ($no. \cdot ha^{-1}$)			Biomass ($kg \cdot ha^{-1}$)		
Section	1982	Mean 1952–61	1956	1982	Mean 1952–61	1956
I	—	—	—	—	—	—
II	443	1 384	741	2.8 (±1.3)	9.7	6.4
III	928	1 594	159	6.8 (±4.4)	9.0	1.4
IV	8 761 (±839)	19 462	31 156	19.3 (±27.3)	71.0	66.2
V	3 765 (±415)	6 437	5 518	15.1 (±24.8)	26.7	20.2
VI	3 868 (±811)	16 687	14 103	17.0 (±66.1)	68.7	35.8
VII	8 197 (±451)	28 103	15 400	26.2 (±20.2)	74.1	54.5
VIII	1 259 (±872)	18 118	20 061	7.9 (±3.9)	49.9	45.1
IX	1 467 (±628)	4 352	3 572	7.9 (±6.9)	13.1	11.8
Section mean	3 586	12 017	11 339	12.9	40.3	30.2

transport rates in excess of 1×10^{-2}, stage = 1.37 m, most of the bed particles would be in motion.

Fish Populations

As mentioned, dead Paiute scuplin of all size classes were collected with coarse bed material during the winter flood of February 1982. These bed-material samples were collected several hours after the flood peak. Hence, the sampled transport rate was significantly less than would have occurred at the flood peak. No further winter floods occurred. In total, 696 individual 4-min samples of bed-material transport were collected at the foot bridge cross-section during the spring floods. No dead fish of any species were ever found in these samples. Occasionally one or two live Paiute sculpins were found in the sediment samples, however. This rate of capture is no more than that expected in the same number of Surber samples (D. C. Erman, pers. observ.).

Based upon measurement at the time permanent fish sampling stations in August 1982, the estimated density of Paiute sculpin ranged from 443 to 8761·ha^{-1} and biomass ranged from 2.8 to 26.2 kg·ha^{-1} (Table 1). Density and biomass were lower in 1982 than the means from 1952 to 1961 ($p < 0.05$, Wilcoxon signed rank test). Density and biomass were also lower in 1982 ($p < 0.05$) than the values for 1956 (the lowest year previously). Only one station (III) had higher values in 1982 than in 1956. At the eight stations where Paiute sculpin were found in 1982, mean density and biomass were 31.6 and 42.7%, respectively, of the 1956 values.

Sampling techniques used for the two studies were different. Furthermore, both sampling techniques are inefficient for collecting young Paiute sculpins. These factors plus the possible serial correlations in the 10-yr data set limit the reliability of tests of statistical significance. Standard errors of population estimates in1982 ranged from 5.8 to 42.1% (mean 11.8%) of the estimates for combined size-classes.

Brook trout YOY (less than 100 mm) were rare at all stations in 1982. Only six individuals were collected from three of the five stations where other age-classes of brook trout were present, giving a range of estimates between 74 and 233·ha^{-1} (mean 79·ha^{-1}; median 88·ha^{-1}). The range of densities for brook trout YOY for the entire creek from 1952 to 1961 was 380 to 4661·ha^{-1} (Seegrist and Gard 1972). The mean density of YOY at the nine stations with brook trout present in 1956 was 397·ha^{-1}. The values for 1982 were significantly lower than either the 10-yr mean or 1956 ($p < 0.05$). It is unlikely that we systematically missed brook trout YOY by electroshocking because we collected rainbow trout YOY, generally smaller than brook trout, in abundance at all stations.

Discussion

There is evidence from several studies in Sagehen Creek that winter floods have been destructive to the age 0 class brook trout (Seegrist and Gard 1972; Erman and Hawthorne 1976; present study). Previous studies (Gard and Flittner 1974) and our current work also show that similar floods reduce the abundance of Paiute sculpin as well. Populations of all ages of rainbow trout in 1982 from sections II–VII were generally near the mean abundance observed from 1952 to 1961 (Erman 1986). Rainbow trout spawn during spring snowmelt runoff. A large percentage of spawners (up to 62%) migrate out of the main channel into small and intermittent tributaries, and they tend to move out before or during the peak flood stage in Sagehen Creek (Erman and Hawthorne 1976). Thus, because of timing and location of spawning, floods result in little disturbance to rainbow trout in Sagehen Creek. Brown trout abundance is relatively low and the effects of flood are difficult to detect.

There are several possible explanations for reduced year-classes or adult fish populations in streams besides flood events. The abundance of fall-spawning Atlantic salmon (*Salmo salar*) parr (1+ and 2+) has been shown to be a function of low discharge during the month preceding spring runoff (i.e. a hatch effect) as well as in the late summer of the year the salmon were born (i.e. a summer growth effect) (Frenette et al. 1984). At least in the case of Paiute sculpin in Sagehen Creek, the observation of dead fish in transport supports the case for winter floods rather than low flow. Nothing is known directly about the fate of brook trout in the present case because the fragile nature of brook trout eggs would make them impossible to identify in sediment samples.

Reductions in fish populations may also occur in winter as a result of short-term flooding from the formation of anchor ice (Maciolek and Needham 1952; Needham and Jones 1959). Mortality occurs when fish are left stranded in dewatered side channels and overbank areas after the ice melts and the waters subside. However, such ice formation is uncommon when heavy snow cover exists (Gard 1963; Chisholm et al. 1987), and winter floods at Sagehen Creek usually occur after a snowpack has been established. Evidence of anchor ice formation can be detected by examination of hourly hydrograph records, as the formation of temporary dams causes a rise in stage for a few hours in early morning (Needham and Jones 1959). We inspected the hydrograph records for Sagehen Creek and found no evidence of frequent or extreme damming attributable to anchor ice formation in the years of rain-on-snow floods. Further, when anchor ice causes mortality, it affects most species and age-classes (Maciolek and Needham 1952; Needham and Jones 1959). In the present case, the cause of mortality must explain both reductions of fall-spawned YOY brook trout and all age-classes of spring-spawning Paiute sculpin. Other mechanisms, of importance in some years at Sagehen Creek, would have a different pattern of effect than that observed from rain-on-snow-caused floods.

We therefore conclude that in Sagehen Creek the agent of fish mortality is the large-scale movement of bed material brought about by exceptional shear forces that develop when stream discharge is confined by deep snowbanks. High discharge during snow-free periods, usually spring, spills onto the flood plain, minimizing additional increases in stream depth and shear force. Only extremely rare floods would be large enough to affect fish populations when the flow is not constrained by an existing snowpack along the channel banks.

A final point that deserves mention is that the impact of snow-constrained floods is not uniform along the length of Sagehen Creek, in part because snow depth is not uniform. Elevational differences significantly influence snowpack. And even within a narrow elevational zone, snow accumulation is patchy. The dominant reason for local patchiness is the type of riparian canopy. Scattered trees and open meadow areas alternate with a dense riparian tree cover throughout the stream length. Snow depth and accumulation is greater in open meadow reaches than under forest canopy, a fact snow hydrologists have used when designing forest clearings to increase snowpack and change the timing of spring runoff (Smith 1974). The relationship among riparian canopy, snow accumulation, and winter floods in Sier-

ran streams points to one more critical role that buffer strips may play in ameliorating the effects of timber harvesting (Newbold et al. 1980; Johnson et al. 1986). Clear cutting to the stream margin or leaving excessively thinned buffer strips may increase the destructive forces of floods during rain-on-snow events.

Acknowledgments

Assistance with fish sampling was kindly provided by Lynn Decker, Nancy Erman, John Hefron, Alison Linden, Diane Shirley, and Bruce Vondracek. Judith McHugh helped with sediment analysis and bedload sampling. We thank Robert Averett, Nancy Erman, John LaBaugh, and William Mathews for reviewing the manuscript.

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Appendix: Calculation of Shear Force and Bed-Material Transport

The transport rate of bed particles in a stream is a function of the fluid forces per unit area, or shear stress, τ, acting on the streambed. Under steady, uniform flow conditions, the shear stress is

$$\tau = \gamma DS$$

where γ is the specific weight of the fluid, D is the mean depth, and S is the water surface slope. The gravitational force resisting particle entrainment, f_g, is proportional to

$$f_g \propto (\gamma_s - \gamma)d^3$$

where γ_s is the specific weight of sediment and d is the particle diameter. The ratio of the fluid forces to the gravitational force is proportional to a dimensionless quantity, called the dimensionless shear stress, τ^*, where

$$(1) \quad \tau^* \propto \frac{DS}{(\gamma_s - \gamma/\gamma)d} .$$

The dimensionless bed-material transport rate per unit width of streambed, Q_B^*, is

$$(2) \quad Q_B^* = \frac{Q_s}{[(\gamma_s - \gamma/\gamma)gd]^{1/2}d}$$

where g is gravity and Q_s is the volumetric transport rate per unit width of streambed determined from bedload samples. The empirical function developed by Parker (1979) is

$$(3) \quad Q_B^* = \frac{11.2\,(\tau^* - \tau_c^*)^{4.5}}{(\tau_c^*)^3}$$

where τ_c^* is the threshold value of τ^* required to initiate particle motion. Equation (3) is essentially identical to the Einstein bedload function (Einstein 1950) but has a much simpler mathematical form. For the bed-material transport curve plotted in Fig. 3, the threshold dimensionless shear stress was computed by a function derived by Andrews (1983) and was found to be in excellent agreement with observed threshold conditions in Sagehen Creek (Andrews and Erman 1986). The computed value of τ_c^* for Sagehen Creek is 0.043. The limits of the curve shown in Fig. 3 are within the range of Einstein's (1950) original data used by Parker (1979).

Evidence for a Hereditary Component in Homing Behavior of Chinook Salmon (*Oncorhynchus tshawytscha*)

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McIsaac, D. O., and T. P. Quinn. 1988. Evidence for a hereditary component in homing behavior of chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 45: 2201–2205.

Returns from three groups of coded wire tagged fall-run chinook salmon (*Oncorhynchus tshawytscha*) in the Columbia River were analyzed to determine the learned and innate components of homing behavior. Homing of an experimental group produced from intercepted adults, reared and released at a hatchery 370 km downstream from the ancestral location, was compared with the homing of the ancestral stock from their natural site and with the stock native to the downriver release site. Controls from the ancestral location and release site homed to their expected destinations with high accuracy. The experimental group demonstrated an intermediate homing response: 58% returned to the release site, 5% returned to hatcheries 12–50 km upstream, 28% were accountable in an intercepting fishery 15–200 km upstream, and 9% returned to the ancestral spawning area, despite total lack of contact with this area.

On a étudié la remonte d'automne de trois groupes de saumon quinnat (*Oncorhynchus tshawytscha*) marqués avec des étiquettes de fil métallique codées dans la rivière Columbia afin de connaître les facteurs acquis et innés du comportement de retour aux eaux ancestrales. On a observé les poissons d'un groupe expérimental produits avec des adultes interceptés, élevés et relâchés à une station de pisciculture située à 370 km en aval des eaux ancestrales, pour comparer leur comportement avec celui des poissons du stock ancestral venant de leur milieu natural et du stock originaire du point de lâcher en aval. Les témoins des eaux ancestrales et du point de lâcher ont atteint la destination prévue avec un grand degré de précision. Les poissons du groupe expérimental sont intermédiaires : 58 % sont retournés au point de lâcher, 5 % se sont rendus à des stations de pisciculture situées à 12–50 km en amont, 28 % ont été retrouvés dans une pisciculture d'interception 15–200 km en amont et 9 % sont retournés dans les eaux de frai ancestrales, bien qu'ils n'y soient jamais allés auparavant.

Received September 28, 1987
Accepted August 9, 1988
(J9440)

Reçu le 28 septembre 1987
Accepté le 9 août 1988

The migratory behavior of salmonid fishes has stimulated a great deal of interest, e.g. in the distances travelled and the accuracy of homing (see review by Brannon 1982). Since the acceptance of the "parent stream theory" (Scheer 1939), various mechanisms have been proposed to explain how salmonids achieve these migrations. Salmon migrations can be divided into three general phases: emigration from freshwater to oceanic feeding grounds, feeding migrations of immature fishes, and the return from feeding grounds to the home stream to spawn. It is generally believed that the first two phases involve innate responses (McInerney 1964; French et al. 1976; Leggett 1977; Hager and Hopley 1981; Wahle et al. 1981). The factors guiding salmon returning from the sea are poorly understood, but the riverine homing phase is generally believed to involve olfactory memory (Hasler and Scholz 1983). However, there is evidence that the progeny of transplanted salmon may not home as accurately as locally adapted stock, implying a heritable component in the final phase of migration (reviewed by Ricker 1972; see Bams 1976; Brannon and Hershberger 1984 for examples).

Studies evaluating the respective roles of learned and innate components in salmonid home stream selection have produced varied results. In a study by Donaldson and Allen (1957) on coho salmon (*Oncorhynchus kisutch*), juveniles reared at one site were transferred to two hatcheries, 50 km apart, in a different watershed for release. Adults returned to the hatcheries of release, not to the ancestral site, suggesting the homing had been based on learned information. However, Bams' (1976) study suggested that there was a genetic component to homing accuracy in pink salmon (*O. gorbuscha*). Salmon with locally adapted paternal genes homed better than pure nonnatal stock, even though survival of the two groups was similar. This paper reports the results of a transplant experiment conducted on chinook salmon (*O. tshawytscha*) in the Columbia River system, in an effort to shed further light on the question of possible genetic components in homing.

Methods and Materials

Study Populations and Sites

The study involved fall-run chinook salmon found in the Columbia River in eastern Washington State, United States (Fig. 1). Two races return to the area above Bonneville Dam

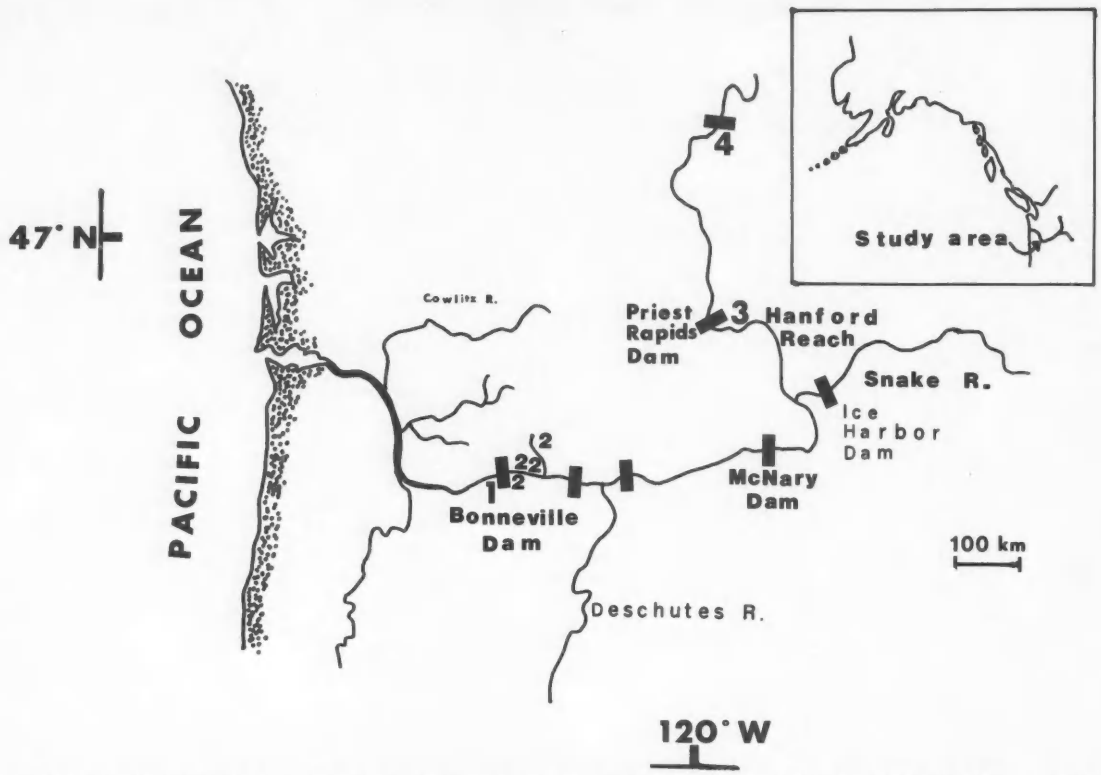

FIG. 1. Columbia River showing (1) Bonneville Hatchery, (2) the four Bonneville Pool hatcheries, (3) Priest Rapids Hatchery, and (4) Wells Hatchery.

TABLE 1. Numbers of tagged fish released, release date, and average size at release for control and experimental groups of 1980 brood Columbia River chinook salmon.

Group	Number of tagged fish released	Release date	Average size at release (g)
URB control	236 600	5–6/81	5.3
Tule control	188 500	5/81	8.2
URB experimental	201 800	6–7/81	10.1

(Howell et al. 1985). One race, locally known as upriver brights (URB), is characterized by bright (oceanic) skin color and lack of development of secondary sex characteristics during its migration over Bonneville Dam. This race primarily spawns on the free-flowing Hanford Reach between McNary and Priest Rapids dams and has smaller populations in hatcheries near Priest Rapids and Ice Harbor dams and a wild natural population in the Deschutes River. The other race, locally known as tules, is characterized by dark skin color and advanced sexual maturity as it passes Bonneville Dam. These salmon originated in the White Salmon River 23 km upstream from the present location of Bonneville Dam, and during the study were additionally produced in four hatcheries on tributaries to the pool above Bonneville Dam and in Bonneville Hatchery, located immediately below Bonneville Dam. Tules have been produced at Bonneville Hatchery for at least 20 generations.

Peak abundance of both races occurs at Bonneville Dam the same time of year (early September), but the URB migration begins sooner and ends later (Pattillo and McIsaac 1982). The tule race spawns primarily during the month of September and spawning is complete by early October; the peak of spawning by the bright race is mid-November but continues at a low level through December (Howell et al. 1985).

Experimental Methods

An experiment, initiated in 1980, permitted evaluation of homing by three groups of hatchery reared chinook salmon: (1) a control group of URB reared at their ancestral location, (2) a control group of tules reared at their natal location about 370 km downstream from the URB site, and (3) an experimental group of URB reared at the same downstream site as the tules. The URB control group consisted of progeny of fish that returned to the Priest Rapids Hatchery area (Table 1). The eggs were incubated and the progeny reared and released at Priest Rapids Hatchery. The tule control group consisted of progeny of fish that returned to Bonneville Hatchery. The experimental group consisted of progeny of the URB race trapped between September 1 and 30 while migrating over Bonneville Dam. Based on unpublished racial verification studies of skin color by the Washington Department of Fisheries in 1979, the possibility of a tule having bright skin color and being misidentified is <5%. The URB experimental group is presumed to be predominantly Hanford Reach origin fish, with some mixture from the other smaller populations. The trapped adult fish were trucked to Bonneville Hatchery, held until sexually mature, and

TABLE 2. Actual and estimated recoveries of tagged experimental and control study groups to areas within the Columbia River, by age of return (ages 2, 3, 4, and 5).

Recovery location	Recovery type	URB control					Tule control					URB experimental				
		2	3	4	5	Total	2	3	4	5	Total	2	3	4	5	Total
Hatcheries and tributaries below Bonneville Hatchery	A[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(%)[b]					(0)					(0)					(0)
Bonneville Hatchery	A	0	0	0	0	0	21	117	23	0	161	170	78	218	51	517
	(%)					(0)					(94)					(58)
Bonneville Pool hatcheries	A	0	0	0	0	0	0	1	0	0	1	22	12	8	5	47
	(%)					(0)					(<1)					(5)
Gillnet fishery between Bonneville–McNary dams	A	1	8	32	13	54	0	2	2	0	4	6	30	51	15	102
	E[c]	2	15	88	37	142	0	4	6	0	10	14	56	141	42	253
	(%)					(27)					(6)					(28)
Hanford Reach natural spawning	A	2	0	6	1	9	0	0	0	0	0	1	0	2	1	4
	E	30	0	98	29	157	0	0	0	0	0	15	0	32	29	76
	(%)					(30)					(0)					(9)
Hatcheries adjacent to Hanford Reach	A	10	53	99	59	221[d]	0	0	0	0	0	0	0	0	1	1[e]
	(%)					(42)					(0)					(<1)
Hatcheries upstream from the Hanford Reach	A	0	0	2	1	3[f]	0	0	0	0	0	0	0	0	0	0
	(%)					(1)					(0)					(0)
Total all areas	E	42	68	287	126	523	21	122	29	0	172	221	146	399	128	894

[a]A = actual recoveries.
[b]Percent of group total.
[c]E = estimated recoveries when subsampling of fishery or escapement areas occurred.
[d]Priest Rapids Hatchery.
[e]Trapped at Ice Harbor Dam, transported to Lyons Ferry Hatchery.
[f]Wells Hatchery.

then spawned. The progeny of these fish were incubated, reared, and released at Bonneville Hatchery and never experienced the environment of their ancestral area or the 370-km downstream migration route between the ancestral area and Bonneville Hatchery. The URB control and experimental groups were released at their typical outmigration time (Allen and Meekin 1973), as was the tule control group (Smith and Wahle 1981).

All salmon received coded wire tags (CWT) unique to each group and were identified externally by excision of the adipose fin. A comprehensive search for returning mature chinook salmon without adipose fins occurred throughout the Columbia River basin during 1982–85 to recover CWT from these and other experimental groups from various hatchery and naturally produced populations. Searching rate varied by fishery, escapement area, and year of return. Fish returning to any hatchery facility in the Columbia River basin were examined for fin clips. Salmon returning to many Columbia River hatcheries, including Bonneville Hatchery and three of the four hatcheries in the Bonneville Pool, enter traps only 100–800 m from the stream mouths that prevent their return to the Columbia River. Basin-wide random sampling of 30–40% of gillnet fishery catches, 5–15% of sport fishery catches, and 3–30% of the salmon reaching natural spawning grounds occurred. Sampling proportions on the Hanford Reach natural spawning grounds ranged from a high of 0.067 in 1982 to a low of 0.032 in 1985. Total CWT present from areas with <100% sampling was estimated by dividing total recoveries by the sampling fraction.

Results

All salmon from all three test groups passed 23 salmon hatcheries located downstream from Bonneville Hatchery during upstream migration; 13 of these hatcheries produced fall-run chinook salmon. All recovered salmon from the URB control group passed Bonneville Dam and were recovered either at their natal site (72%), upstream from their natal site (1%), or were intercepted by the gillnet fishery below the natal site (27%, Table 2). The tule control group returned primarily (94%) to their release site at Bonneville Hatchery, with a single recovery (<1%) in one of the four hatcheries producing tule fish above Bonneville Dam. The remaining 6% of the tule control group were caught in the gillnet fishery above Bonneville Dam. In contrast with the two control groups, 58% of the experimentals were recovered in Bonneville Hatchery, 5% strayed to hatcheries in the Bonneville Pool, 28% were caught in gillnet fisheries between Bonneville and McNary dams, and 9% were estimated to have returned to their ancestral location. Fish from the experimental group migrated over Bonneville Dam in all four return years. A total of five CWT's from the experimental group were recovered in the ancestral spawning location.

The proportion of the experimental group that returned to its release site was significantly smaller than that of the tule control group ($p < 0.001$, chi-square test). Further, the proportion returning to the ancestral location was also significantly smaller than that of the URB control group ($p < 0.001$, chi-square test). The proportion of the experimental fish passing Bonneville Dam estimated to have been caught in the gillnet fishery between Bonneville and McNary dams was significantly greater than that of the URB control group ($p < 0.001$, chi-square test).

The age structure at return for the URB experimental and control groups was typical of URB in general and the age structure of the tule control group was typical of tules. The maturity schedule for the URB race involves a dominant 4-yr-old age

class, with a significant 5-yr-old component; the tule race return typically is dominated by 3-yr olds, with few 4-yr-olds and virtually no 5-yr-old component (Howell et al. 1985).

Discussion

The accuracy of homing in the two control groups was high compared with values reported in the literature. Quinn and Fresh (1984) reported a homing precision rate of 98.6% for spring chinook salmon from Cowlitz River Hatchery, located on a Columbia River tributary below Bonneville Dam. The URB control group passed 20 major tributaries and 38 hatchery facilities to home to the release site and ancestral location at the rate of 99.2% (378 of 381 estimated recoveries in hatcheries and spawning grounds). A single recovery of the URB control group occurred in one of the 13 hatchery facilities above the ancestral location. Ninety-four percent of the tule control group returned to Bonneville Hatchery. This group did not stray to any of the 23 hatchery facilities downstream from Bonneville Hatchery, nor to any of the 19 hatchery facilities above Bonneville Pool. The only stray recovered in a spawning area was in the first hatchery facility (1 km) above Bonneville Dam, although 10 salmon were estimated taken from the gillnet fishery above Bonneville Dam.

If the homing of the URB experimental group had been entirely determined by experience prior to release, they would have returned primarily to Bonneville Hatchery, with perhaps a few caught above Bonneville Dam as was the case with the tule controls. On the other hand, homing based on genetic responses would have brought most fish to the Hanford Reach spawning grounds and Priest Rapids Hatchery, with about 27% intercepted enroute by gill nets between Bonneville and McNary dams. The response of the experimental group was clearly intermediate between these two scenarios. The return of 58% of the experimentals to Bonneville Hatchery but none to the other hatcheries on the lower river suggests that sufficient learning occurred to lead most salmon to their juvenile rearing site. However, the fact that 42% of this group ascended Bonneville Dam contrasts with 6% of the tule controls that did so.

The proportions of the URB control and the experimental groups passing Bonneville Dam that were caught in the gillnet fishery between Bonneville and McNary dams differed from one another. Only 27% of the URB controls were intercepted and 73% reached their natal hatchery, nearby spawning grounds, or stayed upstream. However, 67% of the URB experimentals that passed Bonneville Dam were caught in the gill nets. This information implies that the experimental fish migrated slower or in a less direct manner, something supported by the more protracted pattern of recoveries in the gillnet fishery than the URB control group. The appearance of a confused homing response could be due to the hybridization of the URB populations that comprised the experimental group, as occurred with pink salmon (Bams 1976), as well as conflict between learned and innate responses. Significantly, more experimental fish passing Bonneville Dam migrated over McNary Dam than ascended tributaries, despite the formidable task of negotiating mainstem Columbia River hydroelectric dams (Gibson et al. 1979). It is reasonable to assume that a proportion of these fish could have reached ancestral spawning locations had they not been intercepted by the gillnet fishery.

The four actual recoveries of tagged URB experimental fish on the ancestral spawning grounds were few, because locating marked fish is difficult on the Hanford Reach. However, population estimates for this area, and hence, expansion factors for CWT recovered, are reliable because absolute counts were made of fish at dams above and below this area (United States Army Corps of Engineers 1983, 1984, 1985, 1986; Public Utility District of Grant County 1983, 1984, 1985, 1986). The recovery of experimental fish in three of the four possible return years lends evidence to the conclusion that return to this location was not an aberration.

The learned response of the experimental group is consistent with conventional explanations of salmon homing involving imprinting (Hasler and Scholz 1983). Evidence for genetic influence is contained in the return of some experimental fish to the ancestral spawning grounds and in the intermediate homing response of experimentals compared with the control groups. The four lines of evidence indicating intermediate homing by the experimental group are as follows: (1) the experimental group returned to the release site at a lesser rate than the tule control group, (2) some did return to the ancestral location but at a frequency less than the URB control group, (3) 5% strayed to hatcheries in the Bonneville pool, and (4) they experienced a higher capture rate than the URB control group in gillnet fishing area.

Different types of inherited responses could have influenced the observed behavior of fish in the experimental group. They may have been "programmed" to swim for a distance or a period of time after reentering freshwater exceeding those required to the point of release used in the experiment. They may have had inherited olfactory response to riverine (nonpheremonal) odors of the ancestral area, normally hypothesized to be a learned response for salmon (Hasler and Scholz 1983). There may have been an innate response to population specific pheromones (Nordeng 1977). Inherited preferences for non-site-specific physical criteria such as water temperature and velocity and substrate composition may also have been involved, especially in the extreme terminal migratory phase.

Genetic instructions to swim a long distance after reentering freshwater would be compatible with the sexually immature state (relative to the tule race) of the URB race and the distance to the natal site. Genetic programs for persistence of migratory activity are known in birds (Berthold 1985). However, physiological maturity alone cannot explain upriver migration, as the spring-run chinook that return to the Cowlitz River on the lower Columbia River system typically arrive between March and June, but mature in the Cowlitz River and do not spawn until fall (Quinn and Fresh 1984). The fact that some experimental URB fish returned to Bonneville Hatchery, 370 km short of the ancestral target, does not negate the possibility of genetic instructions for swimming persistence because once trapped at Bonneville Hatchery, salmon were prevented from further upriver progress having been confined to ripening ponds.

There is presently no evidence for population-specific pheromone recognition in chinook salmon, but coho and sockeye (*O. nerka*) salmon can distinguish odors of their population from those of other populations (Quinn and Tolson 1986; Groot et al. 1986). Assuming pheromone recognition, presence of native URB chinook on the upriver spawning grounds and in the Priest Rapids Hatchery while the experimentals were returning allows that population-specific pheromones may have influenced the behavior of those experimentals that ascended Bonneville Dam. However, pheromone recognition alone does not explain the results of this study because no URB controls were decoyed into Bonneville Hatchery by the pheromones of the many experimental URB's present there.

In conclusion, the results obtained do not permit us to determine which factors caused 42% of the experimental chinook to pass their rearing and release site and continue upriver. However, because 9% of the experimental groups accomplished migration to the ancestral site while lacking any experience with the route between that site and their rearing area, a genetically coded response to native habitats and/or population characteristics is indicated. It seems likely that some combination of inherited olfactory response, upstream swimming persistence, and preference for temperature and spawning site characteristics was involved.

Acknowledgements

The authors gratefully acknowledge B. James for technical assistance in data summarization and R. Bams for constructive criticisms of the manuscript. T. Quinn acknowledges NSF Grant BNS8515202 which partially supported his analysis.

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Zinc Influx Across the Isolated, Perfused Head Preparation of the Rainbow Trout (*Salmo gairdneri*) in Hard and Soft Water

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Spry, D. J., and C. M. Wood. 1988. Zinc influx across the isolated, perfused head preparation of the rainbow trout (*Salmo gairdneri*) in hard and soft water. Can. J. Fish. Aquat. Sci. 45: 2206–2215.

At a waterborne [Zn] of 1.9 mg·L^{-1} in hard water (~1 mmol Ca·L^{-1}), Zn influx across an isolated, saline-perfused head preparation of rainbow trout (*Salmo gairdneri*) was about 1.5 nmol·kg^{-1}·h^{-1} through the lamellar pathway and about 1 nmol·kg^{-1}·h^{-1} through the filamental route. Flux rates came rapidly to steady state in both pathways. Trout preexposed to artificial soft water (~0.05 mmol Ca·L^{-1}) for 5 d showed differential stimulation of flux rates to about 42 and 5 nmol Zn·kg^{-1}·h^{-1} through the lamellar and filamental pathways, respectively. Under these conditions, steady-state fluxes across the lamellae did not occur until 15–20 min after the start of perfusion. Preparations from hardwater-acclimated trout tested in soft water gave typical hardwater fluxes showing that these changes in influx were not simply due to acute exposure of the gill surface to low waterborne [Ca]. Influxes in softwater trout, studied over [Zn] from 0.4 to 7.5 mg Zn·L^{-1}, revealed a saturable, first-order uptake with apparent J_{max} and K_m of 150 nequiv·kg^{-1}·h^{-1} and 1.5 mg Zn·L^{-1} (23 μmol·L^{-1}), respectively. Because the apparent K_m is in the toxic range, Zn is clearly not the primary substrate. Scanning electron micrography revealed hypertrophy and increased apical exposure of chloride cells; this stimulation, coupled with the increase in Zn influx, suggests that chloride cells may be the site of entry of Zn across the gill.

À une concentration de zinc dans l'eau de 1,9 mg·L^{-1} dans l'eau dure (environ 1 mmol Ca·L^{-1}), l'influx du Zn à travers une préparation de têtes de truites arc-en-ciel (*Salmo gairdneri*) isolées, après perfusion en solution saline, était d'environ 1,5 nmol·kg^{-1}·h^{-1} dans la voie lamellaire, et d'environ 1 nmol·kg^{-1}·h^{-1} à travers la voie filamentale. Les taux d'influx des deux voies sont rapidement arrivés à l'état d'équilibre. Les truites pré-exposées à de l'eau douce artificielle (environ 0,05 mmol Ca·L^{-1}) pendant 5 d présentaient une stimulation différentielle des valeurs d'influx d'environ 42 et 5 nmol Zn·kg^{-1}·h^{-1} par les voies lamellaire et filamentale, respectivement. Dans ces conditions, des influx à l'équilibre à travers les lamelles n'ont pas été observés avant 15–20 min après le début de la perfusion. Des préparations provenant de truites acclimatées à l'eau calcaire essayées dans de l'eau douce ont donné des influx typiques des eaux calcaires, ce qui monte que ces changements d'influx n'étaient pas simplement dus à une réponse aigüe de la surface des branchies à de faibles concentrations de Ca dans l'eau. Les influx dans la truite d'eau douce, observés à des concentrations de 0,4 à 7,5 mg Zn·L^{-1}, ont mis en évidence une réaction d'absorption saturable du premier ordre avec des valeurs J_{max} et K_m apparentes de 150 nequiv·kg^{-1}·h^{-1} et de 1,5 mg Zn·L^{-1} (23 μmol·L^{-1}), respectivement. Parce que la valeur du K_m apparent est dans la plage toxique, le Zn ne semble pas être le substrat primaire. Par micrographie à balayage électronique, on a mis en évidence l'hypertrophie et une exposition apicale accrue des cellules à chlorure; cette stimulation, combinée à l'augmentation de l'influx du Zn, suggère que les cellules à chlorure pourraient être le point d'entrée du Zn à travers les branchies.

Received December 30, 1987
Accepted July 26, 1988
(J9537)

Reçu le 30 décembre 1987
Accepté le 26 juillet 1988

Zinc (Zn) can be taken up from the water by fish. Although dietary sources may be more important when [Zn] is low in both seawater (Pentreath 1973, 1976; Willis and Sunda 1984) and freshwater (Spry et al. 1988), as waterborne [Zn] increases, so does the importance of this source (Milner 1982; Spry et al. 1988). Despite this, little is understood of the mechanism by which divalent metals traverse the gill. In contrast with monovalent ions, even flux measurements are rare. Branchial calcium (Ca) transport is a recent exception. There are several estimates for unidirectional fluxes, and an understanding of transport mechanisms is beginning to emerge (Hōbe et al. 1984; Flik et al. 1985; Perry and Wood 1985).

Flux rates for trace metals, which are important both nutritionally and as toxic contaminants, have scarcely been examined. Cadmium (Cd) influx rates, studied using a perfused head technique, were extremely low compared with those of monovalent ions and Ca (Pärt and Svanberg 1981). Chelation of the free Cd either decreased or increased influx, depending on the polarity of the resulting chelate (Pärt and Svanberg 1981; Block and Pärt 1986). Copper (Cu) uptake rates in intact rainbow trout (*Salmo gairdneri*) also appeared to be very low, although absolute rates were confounded by adsorption (Laurén and McDonald 1986). Few other metals of environmental significance have been similarly studied.

The mechanism of entry of metals across the gill has generally been assumed to be passive diffusion driven by gradient from water to blood (Pentreath 1973; Rankin et al. 1982). While

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this may be true for metals other than Ca, there are at present few data. Ca, in contrast, appears to be taken up by active mechanisms. Flik et al. (1985) suggested that Ca is actively pumped against a concentration gradient via high-affinity Ca-ATPase in the branchial chloride cell. In accord with enzymatic mediation, this transport was inducible both by exposure to low environmental Ca and cortisol injection and showed saturable kinetics (Perry and Wood 1985). Thus, at least theoretically, two distinctly different mechanisms exist by which metals may traverse the gill.

Our aims were to see if Zn influx across the gill could be detected, and if so, to measure the rate of influx and compare it with those rates which exist for other divalent metals. Second, we wished to gain some insight into the mechanism by which Zn might traverse the gill and to see whether water hardness (i.e. Ca) had any influence on the flux rates. We suspected that there might be some interaction between Zn and Ca, first, because of the well-known action of Ca on permeability, and second, because we have shown that waterborne Zn blocks net Ca uptake and interferes with the plasma Ca regulation in rainbow trout (Spry and Wood 1984, 1985). Third, increased waterborne Ca or Mg decreased Cd transfer across the perfused trout head (Pärt et al. 1985).

Traditional measurements of unidirectional flux rates using radioactive tracer in the environmental water are very difficult to use with divalent cations due to nonspecific adsorption to both the apparatus and the organism. This is true for Ca (Hõbe et al. 1984; Perry and Wood 1985), Cu (Laurén and McDonald 1986), Zn (D. J. Spry and C. M. Wood, unpubl. obs.), and probably other trace metals as well. To circumvent this difficulty, an isolated, perfused rainbow trout head preparation was used. In addition to measuring only that Zn which traverses the gill, use of this preparation allows estimates of lamellar versus filamental contributions to the influx (see Girard and Payan 1980; Payan et al. 1984). Initially, influx measurements were made in hard water, but trout were also preexposed to soft water, since this treatment is reported to induce branchial chloride cell proliferation on the lamellae (Laurent et al. 1985) which appears to be correlated with increased Ca influx (Perry and Wood 1985). The ability to separate lamellar versus filamental routes of uptake allowed us to assess the importance of this proliferation in altering Zn influx.

In the present report, we employ the isolated, perfused head preparation to characterize the uptake of Zn under hard- and softwater conditions and make some assessment of the possible mechanism by which Zn crosses the gill. In the future, this preparation has the potential to elucidate further the mechanism(s) by which Zn traverses the branchial membrane and to determine which waterborne Zn species are available for uptake across the gill.

Materials and Methods

Experimental Animals

Underyearling rainbow trout (297 ± 14 (24) g) were obtained from Spring Valley Trout Farm, Petersburg, Ontario. They were held at McMaster University in flowing, charcoal-dechlorinated, Hamilton city tap water (hardwater (HW): [Ca] 0.9, [Na] 0.6, [Cl] 0.8 mmol·L^{-1}, pH 8.1) at ambient temperature for several months before use and fed a pelleted commercial diet (Martin Feed Mills, Elmira, Ontario). Artificial soft water (ASW) used in some of the experiments was produced by a 15-fold dilution of tap water with either distilled water or water from a reverse osmosis unit. In some experiments, trout were exposed to this medium for 5–7 d prior to experimentation. All trout, regardless of the medium, were temperature acclimated to 15°C at an overall rate not exceeding 1°·d^{-1}. The trout were acclimated in batches of 10–20 in 500-L polyethylene tanks and not fed under these conditions; water was changed on alternate days.

The morphological response of the gill appears to vary with the type of soft water used (Laurent et al. 1985; Perry and Wood 1985). To assess the changes in the surficial tissues of the gill during exposure to the particular soft water used in our experiments, tissue samples were taken from control trout, and trout exposed to ASW for either 1 or 7 d. The second right gill arch was excised and fixed in 2% glutaraldehyde in Cortland saline (Wolf 1963) for 2 h at 4°C, followed by postfixation for 1 h in 1% OsO_4 also in saline. Tissues were dehydrated through an ethanol series, critical point dried out of CO_2, mounted on aluminum stubs, and sputter-coated with gold. Samples were then examined in a Philips 501 scanning electron microscope at 15 keV.

Perfused Head Preparation

Zn influx was measured using the isolated, perfused trout head preparation of Payan and Matty (1975) with various modifications (Perry et al. 1984a; Perry et al. 1985a; Perry and Wood 1985). Briefly, trout were heparinized (2500 USP units i.v.) prior to decapitation. The head was irrigated on an operating table while the gills were cleared of blood with perfusion saline (composition as in Perry and Wood 1985, with 10^{-6} M l-epinephrine bitartrate, 40 g 40 000 molecular weight polyvinylpyrrolidone·L^{-1}, 2 g bovine serum albumin·L^{-1}, fraction V, all from Sigma). A latex diaphragm (condom) was fitted and the head transferred to the flux chamber. The gills were irrigated with 200 mL of either HW or ASW at 200 mL·min^{-1}. Water was aerated and temperature ranged from 14 to 18°C (change <1° during any one experiment). In most experiments, perfusion saline was used. This was gassed with 0.35% CO_2, 4% O_2, balance N_2 to closely approximate in vivo venous gas tensions. Where either whole blood or plasma was required for perfusion, blood was collected from large rainbow trout fitted with indwelling dorsal aortic catheters (Soivio et al. 1972), plugged with stainless steel pins. Blood was drawn as required, pooled, heparinized at a ratio of 200 USP units·mL^{-1}, and gassed as above. Exploratory experiments indicated that dilution to 7% hematocrit with perfusion saline was required to maintain realistic perfusion pressures. Where required, plasma was recovered by centrifugation.

Gills were perfused at constant flow (3.5 mL·min^{-1}, 10 cm H_2O pulse pressure) against a 10 cm H_2O dorsal aortic backpressure, using a Harvard 1405 cardiac pump fitted with a small volume remote head (Davie and Daxboeck 1983). Weight ranges were very similar in all treatment groups, so differences in weight-specific flow were not a complicating factor. Input pressure was monitored continuously with a Hewlett-Packard 267BC transducer, and baseline pressure due to the resistance of tubing and ligatures was subtracted to give the true input perfusion pressure (P_{in}). Data from preparations whose true input pressure did not quickly stabilize at <50 cm H_2O, or which were less than 90% cleared of blood after the experiment, were discarded.

TABLE 1. Types of experiments performed and values for water variables under test conditions; means ± SE.

Series	Perfusate	Exposure	Test	n	Temp. (°C)	pH_w	Ca (mmol·L^{-1})	Na (mmol·L^{-1})	Zn (mg·L^{-1})
1	Saline	HW	HW	7	15.8 ±0.3	7.72 ±0.09	0.98 ±0.02	0.88 ±0.06	1.89 ±0.12
2	Saline	ASW	ASW	20	15.8 ±0.6	7.26 ±0.02	0.05 ±0.02	0.21 ±0.04	Various
3	Saline	ASW	HW	7	14.2 ±0.1	7.78 ±0.50	0.07 ±0.01	0.79 ±0.04	1.99 ±0.05
4	Blood	ASW	ASW	4	14.5 ±0.1	7.33 ±0.02	0.04 ±0.02	0.23 ±0.04	0.61 ±0.14
5	Plasma	ASW	ASW	4	17.0 ±0.6	7.16 ±0.04	0.05 ±0.02	0.23 ±0.04	1.25 ±0.20

TABLE 2. Characteristics of perfusion medium and hemodynamic charcteristics of perfused trout heads.

Series	Perfusate	hct (%)	Differential pressure $P_{in} - 10$ (cm H_2O)	Perfusate flow Q_{tot} (mL·min^{-1})	Branchial resistance R_g (cm H_2O·min·kg·mL^{-1})
1	Saline	0	33.4 ±2.8 (6)	3.21 ±0.05 (6)	2.46 ±0.25 (6)
2	Saline	0	40.4 ±3.5 (9)	3.24 ±0.09 (9)	2.88 ±0.46 (9)
3	Saline	0	29.0 ±1.9 (8)	3.11 ±0.19 (7)	1.68 ±0.22 (7)
4	Blood	7.1 ±1.5 (4)	70.9 ±8.8 (4)	3.21 ±0.17 (4)	4.95 ±0.82 (4)
5	Plasma	0	66.2 ±10.2 (4)	2.76 ±0.16 (4)	4.74 ±1.22 (4)

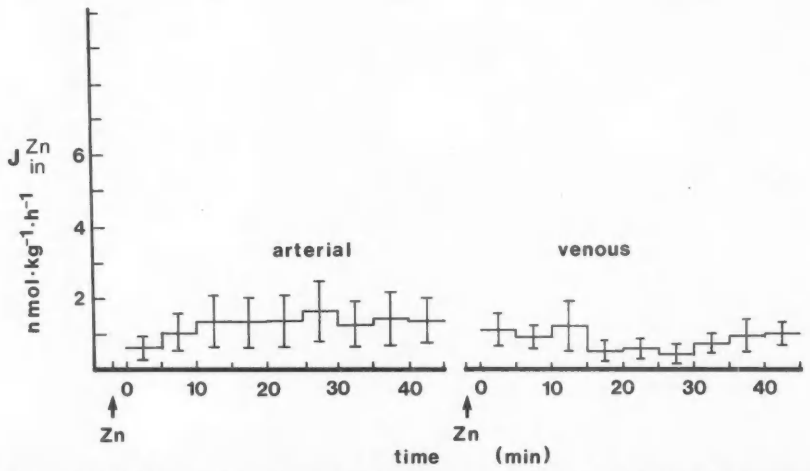

FIG. 1. Zinc influx across the perfused trout head over time. Fish were acclimated to hard water and tested in hard water. Arrows indicate the time of Zn addition. Values are means ± SE, n = 7. Mean external [Zn] was 1.89 ± 0.12 mg·L^{-1}.

When pressure had stabilized (5–10 min), various amounts of Zn (as sulphate) and ^{65}Zn tracer (100 μCi per experiment) were added to the irrigation water and allowed to mix for 2 min before collection of the perfusate began. Perfusate flows from the head preparation were collected in two different streams, the rationale for which was outlined in the introduction. The arterio-arterial flow, passing only through the lamellae, was collected as it dripped from the dorsal aortic cannula whereas the arterio-venous flow, passing through the filaments after the lamellae, was collected from the cut surface of the preparation. Since even small leaks of the irrigation water would contaminate the arterio-venous samples with radioactivity, the refractive index of the arterio-arterial and arterio-venous effluents was carefully monitored with a refractometer (American Optical TS meter). This method was sufficient to detect a leakage constituting only 1% of the arterio-venous flow (Perry and Wood 1985).

Experiments lasted 45–60 min, since this is the optimal life of the preparation. All of the perfusate was collected, in 5-min aliquots, and weighed for flow rate calculations. Water samples (3 mL) were taken concurrently, and replaced with 3 mL of fresh water, to maintain the level in the head chamber. Samples were immediately acidified to 0.2% (water) or 0.4% (perfusate) with high-purity HNO_3 (BDH Aristar). Total [Zn] by atomic absorption spectrophotometry (Varian AA 1275) and ^{65}Zn gamma activity (Chicago-Nuclear well-type counter model 1084) were measured on these water samples whereas perfusate was counted only for gamma activity. For ^{65}Zn activity, samples were adjusted for sample geometry, and since samples were counted within days of the experiments, no decay correction was required.

Experimental Protocol

Five series of experiments were performed (Table 1), all of which examined the time course and rate of influx of Zn into the perfusate. The first series, which utilized saline as the perfusate, assessed influx in trout which were acclimated to the moderately hard Hamilton city tap water (HW). A second series used trout which were preexposed to ASW, with flux rates measured in ASW. Data from this series were used to assess both the time course of uptake, and the effect of varying waterborne [Zn] on influx. Transepithelial potential (TEP) was also measured in these fish using Narco Ag–AgCl electrodes and a Radiometer PHM82 high-impedance voltmeter, as described by Perry et al. (1985b) and Perry and Wood (1985). TEP was used both as a general index of stability of the preparation and to determine whether waterborne Zn affected the electrical gradient for Zn movement across the gills. In the third series, flux measurements were made in ASW, but using HW-acclimated trout. Two other series measured the effect of perfusion with either blood or plasma to see if either of these media would increase Zn influx. In these latter procedures, perfusion commenced with saline and when pressures had stabilized, the perfusate was switched either to plasma or blood. These were performed with fish preexposed and tested in ASW.

Calculations and Statistics

Zn influx rates (nanomoles per kilogram per hour) were calculated using the following equations:

$$(1)\quad J_{in}a = \frac{\text{cpm}\cdot\text{mL}^{-1}\ \text{DA} \times Q_{tot} \times 60}{\text{water specific activity} \times \text{fish weight}}$$

$$(2)\quad J_{in}v = \frac{(\text{cpm}\cdot\text{mL}^{-1}\ \text{AV} - \text{cpm}\cdot\text{mL}^{-1}\ \text{DA}) \times Q_{AV} \times 60}{\text{water specific activity} \times \text{fish weight}}$$

where DA and AV refer to the dorsal aortic (arterio-arterial) and arterio-venous effluents, respectively. Q_{tot} is the total perfusion flow (millilitres per minute), Q_{AV} is the arterio-venous perfusion flow (millilitres per minute), water specific activity is in counts per minute per nanomole Zn, and fish weight is in kilograms.

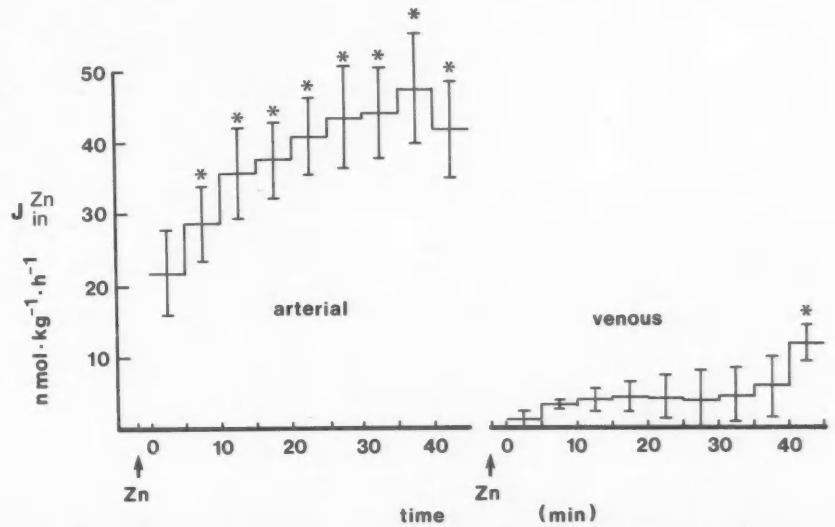

FIG. 2. Zinc influx across the perfused trout head over time. Fish were preexposed and tested in artificial soft water. Values are means ± SE, $n = 6$. Mean external [Zn] was 1.61 ± 0.10 mg·L^{-1}. Asterisks denote means which were significantly different from the first flux period by paired t-test. ($P < 0.05$).

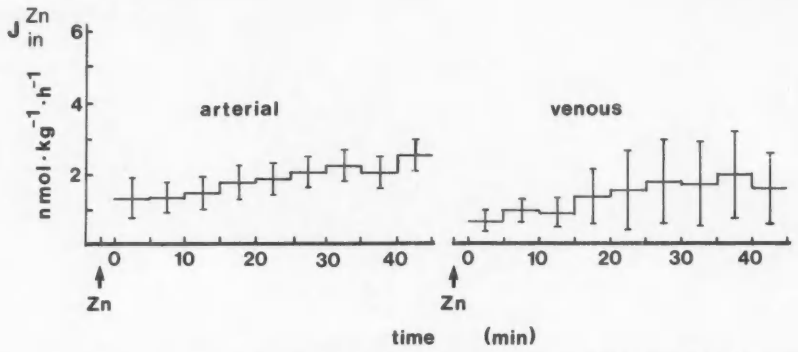

FIG. 3. Zinc influx across the perfused trout head over time. Fish were acclimated to hard water and tested in artificial soft water. Values are means ± SE, $n = 7$. Mean external [Zn] was 1.99 ± 0.05 mg·L^{-1}. There were no significant differences.

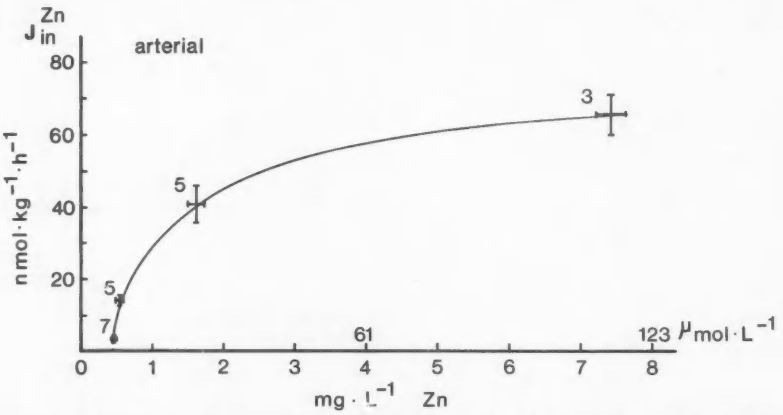

FIG. 4. Zinc influx across the arterio-arterial pathway of the perfused trout head as a function of waterborne [Zn]. Numbers beside each mean denote n.

Mean input perfusion pressure (P_{in}, centimetres H_2O) was calculated as the diastolic pressure plus one-third the pulse pressure. Then:

$$(3) \quad R_g = (P_{in} - 10)/Q_{tot}$$

where R_g is the branchial resistance to flow (centimetres H_2O per minute per kilogram per millilitre), and the dorsal aortic pressure (10 cm H_2O) is subtracted. Q_{tot} here is in millilitres per minute per kilogram.

Mean values ± SE (n) were calculated for each interval for any particular Zn concentration. Statistical significance was calculated by comparing the first interval with subsequent intervals using a paired t-test at the 95% level.

Results

Table 1 summarizes the types of experiments performed and gives the water variables measured. The hemodynamic characteristics for the perfusions are given in Table 2. Ratios of arterial to venous flows (mean 2.8 ± 0.2, $n = 21$) were within the published range for the preparation (Perry and Wood 1985; Perry et al. 1985a). Perfusate recovery was always >90%. The preparation showed good hemodynamic stability and did not show the rapid deterioration seen in some of the preparations used by earlier investigators. In general, with saline perfusion, P_{in} started high, but stabilized quickly at values comparable with those in vivo. Although there were no differences in flow rate among the series, a slightly higher branchial resistance in series 2 resulted in increased input pressure. Use of plasma or diluted blood resulted in a doubling of the branchial resistance and hence P_{in}.

Zn adsorbed readily to the experimental apparatus, such that the external waterborne [Zn] decreased about 25% during the course of a single experiment. The median [Zn] was used as the average value for the experiment, since it gave the same value as the area under the time–concentration curve. Despite this decrease, the specific activity remained constant, so that no correction was required.

In the first series of experiments (HW acclimation, tested in HW, with an external [Zn] of 1.89 mg·L^{-1}), significant influx occurred during the first 5 min of exposure to Zn, in both the arterio-arterial (arterial) and arterio-venous (venous) compartments (Fig. 1). This indicated rapid entry of Zn across the gill. Influx appeared to rise slightly, but not significantly, on the arterial side during the next 5-min interval. Thereafter, influx remained constant. A slight drop in venous influx halfway through the experiment was likewise insignificant. Mean values were about 1.5 nmol·kg^{-1}·h^{-1} on the arterial side and about 1

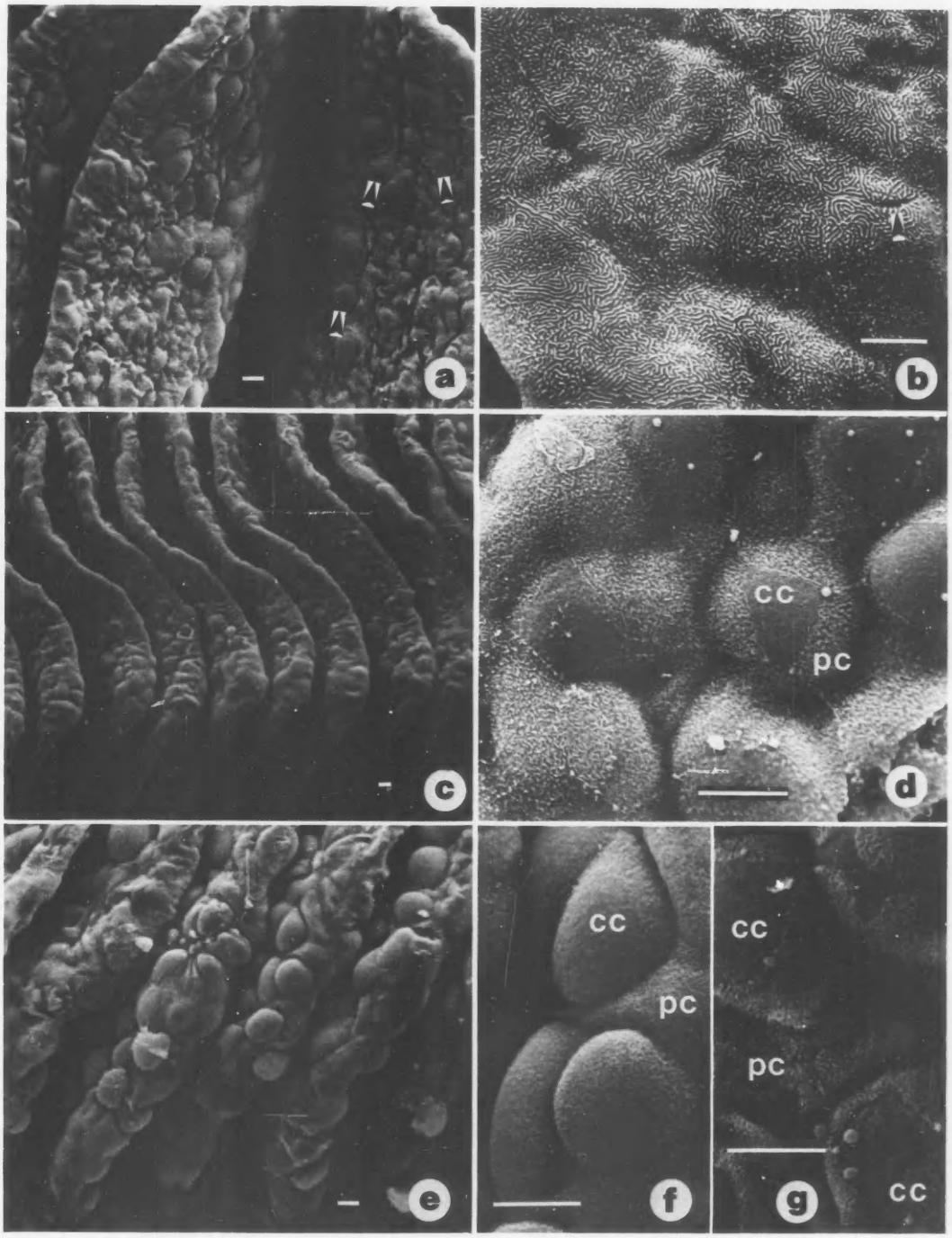

FIG. 5. Scanning electron micrographs of the lamellar surfaces of the gills of rainbow trout. (a) Hardwater control; chloride cells are evident as large bumps. Most are completely overlain by pavement cells; others (arrows) show lenticular-shaped apical exposure. (b) Hardwater control; arrow denotes chloride cell exposure. (c) 1 d of exposure to artificial soft water (ASW). (d) 1 d of exposure to ASW showing pavement cells (pc) and triangular apical exposure of chloride cells (cc) showing some developing microvilli. (e) 7 d of exposure to ASW. (f) 7 d of exposure to ASW showing completely erupted chloride cells. (g) 7 d of exposure to ASW showing overgrowth of chloride cells by pavement cells (upper) and a crenated, degenerating chloride cell (lower right). Scale bars are 10 μm.

nmol·kg^{-1}·h^{-1} on the venous side. Thus in HW, the uptake across the lamellae was only slightly greater than across the filament.

Preexposure and testing in ASW greatly stimulated influx through both arterial and venous routes (Fig. 2). This stimulation was by far more significant on the arterial side. Under these circumstances, J_{in} did not stabilize immediately, but continued to increase, only reaching equilibrium values after 15–20 min. Venous J_{in} stabilized quickly, at much lower levels. Here, J_{in} was significantly increased only during the last interval. It is therefore not clear whether this single high value was anomalous or was the start of an upward trend. Stable values in preparations from ASW-exposed fish were about 42 nmol·kg^{-1}·h^{-1} on the arterial side and 5 nmol·kg^{-1}·h^{-1} on the venous side. In contrast with the HW-acclimated fish, where the arterial contribution was only slightly higher than the venous side, ASW exposure resulted in the arterial side contributing nearly 9 times more Zn than the venous side. Overall, compared with HW acclimation, ASW exposure resulted in 28-fold stimulation in J_{in} on the arterial side and fourfold stimulation on the venous side (compare Fig. 1 and 2).

To shed some light on whether this increase was due simply to the removal of Ca from the gill surface, which is likely to occur during ASW exposure (see below), or to the actual changes in the gill surface which arise from ASW preexposure, the third series of experiments examined flux rates using HW-acclimated fish tested in ASW. The response (Fig. 3) was basically that of the HW trout tested in HW (see Fig. 1). J_{in} tended to rise with time in both arterial and venous pathways, although not significantly, and there were no significant differences between the data sets of Fig. 1 and 3.

The kinetics of branchial Zn influx were measured in ASW-exposed trout by using waterborne [Zn] values ranging from 0.4 to 7.5 mg·L^{-1} (Fig. 4). Since 15–20 min was required to reach equilibrium flux rates in ASW (Fig. 2), kinetics could not be done using several [Zn] values with a single head, as was done previously with Ca (Perry and Wood 1985). Only a single [Zn] could be tested with each head preparation. J_{in} at 0.4 mg Zn·L^{-1} was very low, but thereafter rose rapidly, showing typical first-order saturation kinetics. In fact, there did not appear to be any significant unsaturable influx. It was significant that there was an apparent threshold of 0.4 mg Zn·L^{-1}, below which there was no measurable uptake. This was a departure from first-order reactions, which should pass through the origin. The estimated kinetic constants using arterial data gave an apparent J_{max} of 75 nmol·kg^{-1}·h^{-1} and a K_m of 1.5 mg Zn·L^{-1} (23 μmol·L^{-1}, $n = 20$). Since the venous fluxes were small under these conditions, and followed similar kinetics with increasing [Zn], estimations of K_m and J_{max} using total flux gave nearly identical values.

TEP's were stable with time, varying only 1–2 mV during the course of any one experiment. The total range observed was from −8 to −23 mV, inside negative. TEP was little affected by [Zn]. At the lowest waterborne [Zn] (0.4 ± 0.04 mg·L^{-1}, $n = 7$), the mean TEP was −16.5 ± 1.6 (8). Raising the [Zn] to 7.42 ± 0.21 (3) gave a mean TEP of −10.3 ± 1.2 (3), a change which was not significant.

Perfusion with either plasma or diluted whole blood was done to determine if these natural fluids provided additional substances (ligands) which would enhance J_{in}. Despite the presence of red blood cells and/or increased plasma protein (as determined by refractive index), influx was actually less than that found with perfusion saline, although individual values were quite variable. For blood diluted with perfusion saline to a hematocrit of 7% (Table 2), arterial J_{in} was 2.4 ± 0.8 (4) nmol·kg^{-1}·h^{-1} at a waterborne [Zn] of 0.61 ± 0.14 mg Zn·L^{-1}. For undiluted plasma, arterial J_{in} was 19.7 ± 4.2 ($n = 4$) at a waterborne [Zn] of 1.25 ± 0.2 mg Zn·L^{-1}. Comparable values for saline perfusion from the influx curve of Fig. 4 would be about 18 nmol·kg^{-1}·h^{-1} at 0.6 mg Zn·L^{-1} and 35 nmol·kg^{-1}·h^{-1} at 1.25 mg Zn·L^{-1}.

Examination of the gill tissues by scanning electron micrography revealed that under control conditions (Fig. 5a, 5b), chloride cells were clearly visible on the lamellae as large bumps which tended to be more numerous toward the trailing (downstream) edge. The cells were completely overlain by pavement cells, or nearly so, with only occasional lenticular exposure of the apical surface (Fig. 5b). Under these conditions, the apical surfaces of the chloride cells did not show the microvillar and microridge structure which was typical of pavement cells. After 1 d of exposure to ASW, little difference was seen at low magnification (Fig. 5c). At higher magnification, however, exposed apical surfaces were triangular in shape and clearly larger in area (Fig. 5d). Surfaces of chloride cells often appeared stippled with microvilli. When exposure to ASW extended to 7 d, profound changes occurred in lamellar chloride cells which were obvious even at lower magnification (Fig. 5e). Chloride cells were much more numerous and showed three distinct types representing a progression of stages. The most common chloride cells were quite rounded, and erupted from the lamellar surface. The apical surfaces frequently had well-dveloped microvilli and microridges with little, if any, pavement cell encroachment (Fig. 5f). Other chloride cells showed varying degrees of overgrowth by pavement cells, and in a few instances the chloride cells themselves appeared degenerate, having shrunken apical surfaces (Fig. 5g). Complete coverage by pavement cells at any stage was rare.

Discussion

Zn influx was successfully measured under all conditions of acclimation and testing using the perfused head preparation. In addition, further information was obtained concerning the contribution by arterial and venous pathways. The rationale for the partitioning of perfusate flow through the head was described by Girard and Payan (1976, 1980) and Payan et al. (1984). The arterial flow is quite straightforward, being that perfusate which is collected from the dorsal aorta, and is considered to represent flux through the lamellae. However, assumptions underlying the venous flow and its representation of filamental fluxes are somewhat more complex. Venous flows in the gill are considered to arise from oxygenated blood which has already transited the lamellae and entered the filamental venous sinus (FVS) where it is in close contact with the filamental epithelium. The FVS then drains via the branchial veins back to the ventricle. Discrete sampling of this drainage would indeed give a measure of filamental input, simply by subtracting the arterial component. However, other fluids may also find their way into this pool. There is venous drainage which was never in contact with the FVS, namely venous drainage arising from systemic arterial flow to the brain and musculature of the head. There is also the possibility of arterial flow from arterioles at the cut surface. This extraneous drainage into the arterio-venous collection does not alter the calculation of filamental uptake, as long as no Zn is lost in the systemic circulation prior to collection. However, Zn is cleared quite quickly to the tissues from the systemic

circulation in vivo (D. J. Spry and C. M. Wood, unpubl. data) although extent of "first pass" clearance (similar to the situation with the perfused head) is unknown. Thus the venous values could underestimate filamental Zn influx. A second possible cause of underestimation of the venous influx might arise from lack of true venous steady state. This was suggested, but not proven, by Fig. 2. Similarly, Gardaire et al. (1985) have discussed the circuitousness of the venous circulation and the time lag for isotopic steady state between the arterial and venous circulation with regard to Na flux. The opposite possibility, that of overestimates of venous flux due to contamination from the irrigation water, was described by Perry and Wood (1985). Large leaks, mainly through the esophageal ligature, were detected readily by refractometry in the present study and either rectified or the data discarded. Small leaks (<1%) cannot be completely ruled out, and given the high specific activity of the water, these would contribute significantly to the venous flux. However, the constancy of the fluxes over time (Fig. 1–3) and the rapid saturation with increasing [Zn] (not shown, but similar to the arterial data of Fig. 4) argue against even small leaks. On balance we conclude that the arterial (lamellar) Zn uptake estimates in our experiments are valid, while the venous (filamental) uptake estimates are either valid or underestimates.

Zn fluxes in HW-acclimated and tested trout came quickly to steady state with minimal time lag and were the same for both arterial and venous fluxes. The flux rates, in the low nanomoles per kilogram per hour range, were comparable with those of Cd at similar exposure concentrations (Pärt and Svanberg 1981). However, they were small compared with flux rates for Ca which have ranged from 2 to 50 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ in vivo (Berg 1968; Pang et al. 1980; Mayer-Gostan et al. 1983; Hõbe et al. 1984; Perry and Wood 1985) and using perfused gill preparations (Payan et al. 1981; Perry and Wood 1985). At least in HW, Zn did not exhibit the disparity between arterial (90–95% of total) and venous fluxes reported by Perry and Wood (1985) for Ca. The similarity of arterial and venous Zn fluxes suggests that Zn uptake per unit area (specific uptake) was greater through the filament than through the lamella, since lamellar area in rainbow trout is about 27-fold higher than filamental area (Girard and Payan 1980). Chloride cell density is much higher in the filament, especially in hard water (Laurent and Dunel 1980; D. J. Spry, unpubl. obs.), which may account for the higher apparent specific uptake (see below).

Zn fluxes in fish preexposed and tested in ASW were stimulated more than 20-fold above HW rates. This increased uptake may at least partially explain the well-known fact that Zn toxicity increases with decreasing water hardness (Spear 1981). Furthermore, the Zn uptake in ASW differed in two other ways from that in HW. First, steady-state flux rates did not occur in the arterial perfusate until 15–20 min after addition of the isotope. Second, the venous influx, although stimulated, was considerably less than the arterial influx. Unless the relative surface areas of the lamellae and the filament changed drastically, this would suggest that the Zn uptake per unit area across the lamellae increased much more than through the filament. However, the increase in venous influx during the last flux period (Fig. 2) may again indicate a long time lag in Zn flux through this compartment.

The TEP's measured under these conditions were similar to those measured in other freshwater-adapted teleosts (see Potts 1984; Perry et al. 1985b; Perry and Wood 1985). Since this electrical potential is, in freshwater fish, largely the result of Na^+ and Cl^- diffusion, the stability of the TEP both over time and across [Zn] indicated relative permeabilities for Na^+ and Cl^- which were also stable. This agrees with earlier in vivo work of Spry and Wood (1985) which indicated that Zn effects upon Na^+ and Cl^- were minimal compared with other toxicants such as acid or Cu (McDonald 1983; Laurén and McDonald 1986).

The stimulation of Zn influx by exposure to ASW and the preferential effect on the lamellar pathway were clearly due to more than the removal of Ca from the water (and gill surface), as was demonstrated by the experiments of series 3. These results strongly suggest that actual morphological and/or functional changes occurred in the gill, which were responsible for this increased flux. Recent work has demonstrated that many stresses, and in particular, exposure to water low in Ca (Perry and Wood 1985) or NaCl (Laurent et al. 1985) induced changes in the gill epithelium. Not only did the number of chloride cells on the lamellae increase, but the overlying pavement cells receded, so that more of the cell was exposed to the environment (Perry and Wood 1985). We also found (Fig. 5) that simultaneous dilution of both NaCl and Ca induced similar changes in branchial chloride cells. In addition, there was dramatic development of microvilli and microridges. While this undoubtedly further increased surface area, the functional significance is less clear. Although the changes in chloride cells in terms of number, distribution, exposure, and surface structure do not show causation, the correlation strongly suggests a role for the chloride cell in the uptake of Zn from the environment. In contrast, Pentreath (1973) failed to find any localization of ^{65}Zn in autoradiographs of chloride cells from seawater plaice (*Pleuronectes platessa*), but the Zn concentration was much lower ($0.015\ mg \cdot L^{-1}$).

The reason for the long time lag to steady-state Zn flux in ASW-exposed trout is unclear. Pärt and Svanberg (1981) reported similar time lags to steady state for Cd, even though their work was done in hard water. Perhaps unstirred layers on the gill increased the diffusion distance sufficiently to slow the rate of arrival of metal at the site of entry on the gill. These layers may have arisen from increased mucus production as a result of ASW exposure (in our experiments) or from Cd exposure (in Pärt and Svanberg's experiments). Mucus also bound Cd and Hg, and while it did not impede the movement of Ca, diffusion of Cd and Hg was significantly slower (Pärt and Lock 1983). If Zn were trapped by anionic sites in mucus and the gill surface, this might result in the observed time lag. This phenomenon might also explain the apparent threshold of ~0.4 mg $Zn \cdot L^{-1}$ below which there appeared to be no influx. Longer exposure times might, however, result in detectable influx.

The experiments with plasma and blood perfusion were performed to test whether Zn uptake by the perfused head was limited by the Zn binding capacity of the perfusate, i.e. whether the provision of organic ligands in plasma protein and red blood cells would stimulate influx. The results showed that this was clearly not the case. Influx in fact, was considerably lower, probably due to increases in the branchial resistance (Table 2) indicative of altered hemodynamics with blood and plasma.

We initially proposed two possible mechanisms by which Zn might traverse the gill: simple passive diffusion or active uptake. The electrochemical gradient probably favours the passive entry of Zn into the fish. This is so first, because the inside of the preparation had negative potential (TEP −8 to −23 mV inside negative in ASW). Second, at this water pH, most of the Zn exists as hydrated free ion in the water (Campbell and Stokes 1985) whereas in blood and other biological fluids, Zn is tightly

bound, with very little existing as free ion (Hambidge et al. 1986). The kinetic data suggest that passive influx, in which flux would be a simple linear function of external concentration, was of very minor importance if it occurred at all. The curvilinear nature of the influx–concentration relationship supports a saturable uptake mechanism, although the mechanism is not necessarily active or even carrier mediated.

One mechanism which might give the appearance of saturable influx is passive diffusion being reduced by an increase in diffusion distance with increasing waterborne [Zn]. High waterborne concentrations of Zn cause inflammation and edema of gill tissue in vivo (Skidmore and Tovell 1972; Tuurala and Soivio 1982; Tuurala 1983). Could edema result in a concentration-dependent fashion with increased waterborne [Zn], and could this masquerade as a saturable phenomenon? First, the preparation itself is free of any significant edema (Perry et al. 1984a, 1984b) using current methodology. The edema associated with waterborne Zn exposure, on the other hand, resulted from its corrosive action as an external irritant. This took 2 h of exposure at a very high level (40 mg $Zn \cdot L^{-1}$; Skidmore and Tovell 1972) and 96 h of exposure to 1.25 $mg \cdot L^{-1}$ (Tuurala and Soivio 1982) for pathology to occur. Decreases in Pa_{O_2} indicative of increased diffusion distance, occurred after 6 h of exposure to ~1.5 mg $Zn \cdot L^{-1}$ (Sellers et al. 1975; Spry and Wood 1984). Tuurala (1983) correlated increased difference between inspired water P_{O_2} and Pa_{O_2} with increased diffusion distance after 17–20 h of exposure to 1 mg $Zn \cdot L^{-1}$. The brief period of perfusion in the present study, and the lower [Zn] used, precludes this as a significant factor. Further evidence against edema is that there was never a decrease in influx rate over time, as would be expected if edema were creating a diffusion limitation. The saturability of influx must thus be due to other factors, the most likely of which is a saturable carrier, or a selective pore. It is not possible to distinguish between these possibilities based on the present data. What is readily apparent from the kinetic constants, however, is that the K_m is well into the toxic range for trout (2.5–3 $mg \cdot L^{-1}$; Holcombe and Andrew 1978) and thus Zn transport is coincidental. Such "accidental" uptake by a Ca mechanism was suggested to occur in freshwater amphipods (Wright 1980).

Our results have clearly shown that significant uptake of Zn occurred from the water and indicate a mechanism by which this transfer is accomplished. Actual transfer rates for Zn may be considerably higher in vivo based on two facts. First, uptake from moderately hard water, when dietary Zn was deficient, was about 120 $nequiv \cdot kg^{-1} \cdot h^{-1}$ (Spry et al. 1988), and whole-body influx based on ^{65}Zn kinetics in vivo (D. J. Spry and C. M. Wood, unpubl. data) was 200–400 $nequiv \cdot kg^{-1} \cdot h^{-1}$. Nevertheless, this preparation has yielded important qualitative understanding of the mechanism involved in transbranchial Zn transfer. Even these higher rates are small (1%) compared with those of other ions such as Na, Cl, and Ca. Excretion of Zn, however, is also very low (Pentreath 1973, 1976; Willis and Jones 1977). Estimates for elimination half-times were 235 d for the largest of three compartments (91%) in *Gambusia* (Willis and Jones 1977) and about 300 d for plaice (Pentreath 1973), both in seawater. Coupled with the fact that Zn was taken up independently from both the water and the diet (Spry et al. 1988), elevated waterborne concentrations could cause significant branchial uptake and retention.

In conclusion, the saline-perfused head was a useful model to demonstrate entry of Zn across the branchial epithelium of trout and gain some insight into relative importance of lamellar and filamental pathways at different hardness. Use of this method permitted measurements of influx which were relatively free from the confounding effects of metal adsorption. Zn uptake rates were extremely low (0.01–1% of Na, Cl, and Ca influx rates). Use of blood or plasma as perfusates did not augment Zn uptake. ASW exposure greatly stimulated Zn influx, especially through the lamellae, and this effect likely arose from morphological changes in the gill. Changes in chloride cell number and distribution were the most likely explanation.

Acknowledgements

We are particularly indebted to Dr. S. F. Perry for his instruction in the technique of isolated head perfusion. Large trout were a gift of Dr. A. J. Niimi, Canada Center for Inland Waters. Mrs. J. Carson helped greatly with the electron microscopy. Dr. D. J. Laurén is thanked for discussions of gill morphology. The work was supported by an NSERC Strategic Grant in Environmental Toxicology to C.M.W.

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Reconstruction of Harvest Rates and Stock Contribution in Gauntlet Salmon Fisheries: Application to British Columbia and Washington Sockeye (*Oncorhynchus nerka*)

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Starr, P., and R. Hilborn. 1988. Reconstruction of harvest rates and stock contribution in gauntlet salmon fisheries: application to British Columbia and Washington sockeye (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 45: 2216–2229.

We describe a run reconstruction method in which information on migration paths and travel speeds is combined with catch and escapement data to estimate harvest rate by fishery and catch by stock for Pacific salmon. Reconstruction is shown to provide comparable estimates to direct stock identification from scales in the example given. Methods of annual reconstruction are shown to be less reliable than weekly or half-weekly period reconstructions when exploitation rates change during the season. Equations are provided for incorporating differential vulnerability of stocks, and Monte Carlo methods for calculating confidence limits on harvest rate estimates are explored.

Les auteurs décrivent une méthode de reconstitution des remontées du saumon du Pacifique qui repose sur la réunion de données sur les voies de migration et les vitesses de déplacement avec des données sur les prises et les échappées pour l'estimation des taux de récolte des pêches et des prises par stocks. Les auteurs montrent, par l'utilisation d'un exemple, qu'il est possible d'obtenir des estimations comparables à celles fournies par l'identification directe des stocks à partir des écailles. Ils montrent aussi que les reconstitutions annuelles sont moins fiables que celles portant sur des périodes de l'ordre d'une semaine ou d'une demi-semaine lorsque les taux d'exploitation varient au cours de la saison. Ils présentent des équations permettant de tenir compte de la vulnérabilité différentielle des stocks et traitent de méthodes de Monte Carlo pour le calcul des limites de confiance des taux de récolte estimés.

Received March 17, 1987
Accepted August 22, 1988
(J9180)

Reçu le 17 mars 1987
Accepté le 22 août 1988

The majority of Pacific salmon are captured on the west coast of North America by a number of fisheries in which the maturing fish are caught as they migrate towards their natal streams. These gauntlet fisheries may take place in the natal streams, or hundreds of miles away when the fish first come close to shore. Successful implementation of any harvest strategy requires an understanding of the harvest rates that occur in each fishery and the contribution of different stocks to the catches of each fishery.

Estimates of the harvest rates are needed to determine fishing plans and to evaluate the effectiveness of alternative regulations and past management actions. In-season run size estimates often depend upon assumed harvest rates, and these must be calibrated from past data.

The determination of contribution by stock to catch is needed to determine total production from each stock. Calculation of stock and recruitment relationships requires estimates of total brood year production, which in turn requires the separation of different stocks within mixed fisheries. International agreements such as the allocations by the Pacific Salmon Commission (PSC) require estimates of contribution of different stocks to various fisheries.

There are two basic approaches to the separation of stock contributions: one is direct identification of stream of origin by tags, morphology, electrophoresis, stock-specific parasites or scales; the second method uses escapement data, catch data, and knowledge of migratory paths and timing to calculate stock contribution and harvest rates. This second method is known as run reconstruction and is currently practiced by several salmon management agencies on the west coast of North America. This paper describes the theory and practice of run reconstruction and assesses its limitations.

Despite the fact that salmon managers have used run reconstruction for at least 50 yr, there is no literature describing the method or examining its limitations. A run reconstruction method is described in Palmer (1972), but the method is not described in sufficient detail to be replicated. Schnute and Sibert (1983) have described a sophisticated analysis of a terminal fishery in which they reconstruct harvest rates, among other parameters. Their paper, however, is concerned with a single terminal fishery with no stock separation problems and therefore addresses much simpler problems.

In this paper we will describe the run reconstruction methodology as practiced in British Columbia and Washington. We will provide some examples of specific analyses and compare the results of run reconstruction with those obtained by other methods. We will describe the assumptions of the methodology

TABLE 1. Annual reconstruction of the 1978 pink salmon fishery in Statistical Area 8 (Fisher Fitzhugh Sound, Bella Coola) of British Columbia.

Area	Number of fish	No. present before the fishery	Harvest rate
Bella Coola escapement	2 293 000		
Gillnet area catch	510 000	2 803 000	0.18
Outside fishery Fitzhugh Sound catch	1 174 274	3 977 274	0.30
Total run	3 977 274		

TABLE 2. Hypothetical reconstruction of Fraser River and Lake Washington sockeye. All catches and escapements in thousands.

Stock	Escapement	Fishery	Catch	Harvest rate
Fraser River	840	Fraser River	209	0.20
Lake Washington	159	San Juan Islands	472	0.28
		Juan de Fuca Strait	720	0.30

	Stock		
Fishery	Fraser River	Lake Washington	Total
	No. after fishery (P_{ij})		
Fraser River	840		
San Juan Islands	1049	159	1208
Juan de Fuca Strait	1459	221	1680
	No. before fishery (N_{ij})		
Fraser River	1049		1049
San Juan Islands	1459	221	1680
Juan de Fuca Strait	2084	316	2400

and discuss the validity of these assumptions. We present some methods for quantitatively determining the sensitivity of our results to uncertainty in the data and finally discuss the role of reconstruction in salmon management.

Single Time Period Reconstruction

One Stock, One Fishery

In its simplest form, run reconstruction has been used by salmon managers since catches and escapements were first measured. It relies on the following simple relationships:

$$N = C + E$$
$$h = \frac{C}{C + E} \quad (1)$$

where N is the total return of the stock, C is the catch, E is the escapement, and h is the harvest rate.

One Stock, Several Fisheries

When a stock passes through a gauntlet of fisheries, we may want to know the harvest rate exerted in each of n fisheries. To reconstruct these harvest rates, assuming the final escapement E is known, consider the following algorithm:

$$P_j = E \quad \text{if } j = n$$
$$P_j = N_{j+1} \quad \text{if } j \neq n$$
$$N_j = P_j + C_j \quad (2)$$
$$h_j = \frac{C_j}{N_j}$$

where P_j is the number of fish surviving the jth fishery, N_j is the number of fish available to the jth fishery, E is the escapement, C_j is the catch in the jth fishery, and n is the number of fisheries.

The actual calculation of equation 2 should be done backwards from $j = n$ to $j = 1$. Table 1 shows a simple reconstruction of pink salmon (*Oncorhynchus gorbuscha*) in Statistical Area 8 of British Columbia in 1978. The fish first enter a dominantly purse seine fishery in the outside portion of area 8 (Fisher Fitzhugh Sound) and then pass into a gillnet fishery near Bella Coola. By working backwards through the fisheries, one can easily arrive at the harvest rate in each fishery.

Several Stocks, One Fishery

If we assume that each stock is equally vulnerable in our fishery (an assumption that is modified later), the simple reconstruction equation 1 can be slightly modified to yield

$$N_i = E_i + C \frac{E_i}{\Sigma E_i}$$
$$h = \frac{C}{C + \Sigma E_i} \quad (3)$$

where N_i is the total return of stock i, E_i is the escapement of the ith stock, C is the catch, and h is the harvest rate.

Again this is a rather trivial case that simply assumes that the catch contribution of each stock is proportional to its escapement.

Several Stocks, Several Fisheries

When we treat our data on an annual basis, using total catch by fishery (C_j) and escapement by stock (E_i) and we also specify the path that each stock follows through the different fisheries, we can perform a reconstruction of the catch contribution to each fishery. As a simple example, consider two stocks, Fraser River sockeye (*Oncorhynchus nerka*) (Canadian) and Lake Washington sockeye (U.S.). There are three fisheries: (1) Canadian Juan de Fuca Strait, (2) U.S. San Juan Islands, and (3) Fraser River. Fraser River fish pass through all three fisheries; U.S. fish only go through the Juan de Fuca Strait and San Juan Islands fisheries.

We need a notation to express the migration path of each stock. Let us designate $O_{i,o}$ to be the fishery the ith stock is in its oth area. Further let NO_i be the number of areas the ith stock passes through. Thus if stock 1 is the Fraser River and stock 2 is Lake Washington, then $NO_1 = 3$ and $NO_2 = 2$. Further $O_{11} = 1$, $O_{12} = 2$, $O_{13} = 3$, $O_{21} = 1$, and $O_{22} = 2$.

We can now write our reconstruction equations as follows:

$$P_{i,o_{ij}} = E_i \quad \text{if } j = NO_i$$
$$P_{i,o_{ij}} = N_{i,o_{i,j+1}} \quad \text{if } j \neq NO_i$$

$$(4)\quad N_{ij} = P_{ij} + \frac{C_j P_{ij}}{\sum_i P_{ij}}$$

$$h_j = \frac{C_j}{\sum_i N_{ij}}.$$

Table 2 shows a hypothetical reconstruction of this fishery.

Multi-time-period Reconstruction

Most gauntlet fisheries for salmon occur over several weeks with the fishing effort changing considerably during that period. There will normally be a few days per week of fishing permitted, and many days per week without any fishing. Fishing effort may be high during some weeks and low during others. Because of changing fishing efforts, it is often unrealistic to assume that harvest rates are the same on each stock. If one stock has a later run than another and the effort is concentrated at the beginning of the season, then the early stock will be exploited at a higher rate than the later stock. If we break the reconstruction into discrete time periods, we can estimate the differential harvest rates as the season progresses.

Within-season management generally requires an understanding of the relationship between fishing effort, fish abundance, and realized harvest rate on catch. This requires a reconstruction of each fishing period, not a total annual reconstruction. For these reasons, we have developed a general multi-time-period method of reconstruction that is analogous to our annual reconstruction and adds the requirement of needing catch by time and area, escapement by time and stock, and the residence time of each stock of fish in each harvest area.

Now, we discuss only the theoretical data requirements. Later, we consider the practical limitations of data availability.

Multi-time-period reconstruction can operate on any time scale: months, weeks, days, or even hours. In practice, limitation of catch and escapement data usually leads us to reconstruct on a weekly, half-weekly, or daily basis. The major constraint is that the spatial scale must be large enough that the fish spend a minimum of one time period in each area they pass through. Thus, for weekly reconstruction, the areas must be large enough to encompass 1 wk of migration distances. This will often lead to aggregation of catch areas, or to using a relatively short time interval.

The underlying movement model assumes that each fish in a stock migrates through a gauntlet of fisheries and spends a certain number of time intervals in each fishery. Each fish of the same stock must spend as many days in each area as did all other members of the same stock. For example, we might believe that Fraser River fish spend 3 d in the Juan de Fuca fishery, 5 d in the San Juan Island fishery, and 7 d in the Fraser River fishery. Such data are often obtained from adult tagging studies. The assumption of the reconstruction method is that every Fraser River bound fish will spend 3 d in Juan de Fuca, 5 d in San Juan Island, and 7 d in the Fraser River.

Thus we assume that every Fraser River bound fish will pass into the escapement 15 d after it first enters the Juan de Fuca fishery if it is not caught in an intervening fishery. Denoting the fisheries as 1 = Juan de Fuca, 2 = San Juan Island, and 3 = Fraser River, we would describe the movement path of Fraser River fish as 1-1-1-2-2-2-2-2-3-3-3-3-3-3-3. If we felt that Lake Washington bound fish spend 3 d in the Juan de Fuca Fishery and 7 d in the San Juan Island fishery, their migration path would be 1-1-1-2-2-2-2-2-2-2.

It is convenient to think of each stock as a series of individual "clumps" of fish that enter the first fishery together on the same day and travel together day by day, some members of the clump being harvested along the way. In our example above, the San Juan Islands fishery would contain at any time five clumps of Fraser River bound fish and seven clumps of Lake Washington bound fish.

We need the following notation:

E_{ik} = escapement of ith stock during the kth time period, C_{jk} = catch in jth area during kth time period, O_{io} = number of the fishery where a clump of the ith stock is in o time periods after entering the first fishery, ns = number of stocks, na = number of areas, np = number of time periods, and NO_i = number of time periods a clump of the ith stock takes to pass from the first area into the escapement.

The following will be calculated: N_{ijkl} = number present before the fishery of the ith stock, in the jth area, during the kth time period, of the clump which entered the first fishery this stock passes through on the lth time period, P_{ijkl} = number of fish after the fishery (subscripts are the same as for N), T_{jk} = total number of fish present before the fishery in the jth area, kth time period, and h_{jk} = harvest rate in the jth area during the kth time period.

Given the notation described above, and the basic assumptions that each fish of a particular stock takes the same number of time periods in each area as does every other fish in the same stock and that all fish are equally vulnerable in any area, we can compute N, P, T, and H as follows:

$$(5)\quad \begin{aligned} P_{ijkl} &= E_{i,k+1} \qquad \text{if } j = O_{i,NO_i} \\ P_{ijkl} &= N_{ij'\,k+1,l} \qquad \text{if } m \neq O_{i,NO_i} \end{aligned}$$

given that

$$j = O_{im}$$
$$j' = O_{i,m+1}$$

and further:

$$N_{ijkl} = P_{ijkl} + C_{jk}\left[\frac{P_{ijkl}}{\sum_{i=1}^{ns}\sum_{l=1}^{np} P_{ijkl}}\right]$$

$$T_{jk} = \sum_{i=1}^{ns}\sum_{l=1}^{np} N_{ijkl},$$

$$h_{jk} = \frac{C_{jk}}{T_{jk}}.$$

These equations must be solved by moving backwards over the time subscript (k) and then updating P, N, T, and h for all i, j, and l.

Sample Reconstruction of 1982 Sockeye Run to Southern British Columbia

The reconstruction of the 1982 sockeye run to southern British Columbia and Washington is presented as an example of a multistock, multi-time-period analysis. This reconstruction is described in more detail elsewhere (Starr et al. 1984). Included in the analysis are all sockeye catches and escapements occurring in southern British Columbia and Puget Sound south of Cape Caution (latitude 50°09′, Fig. 1). The only exceptions are the sockeye stocks in Barkley Sound off the west coast of Vancouver Island which are harvested in a local terminal fishery and do not contribute to the catches considered here.

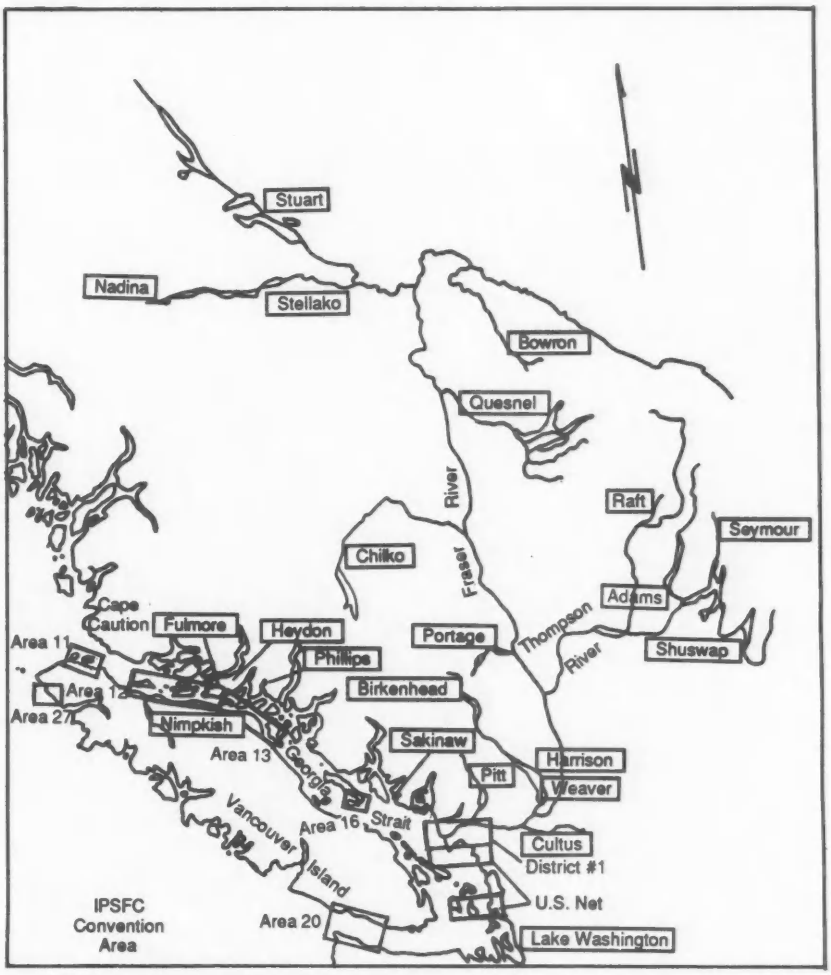

FIG. 1. Major sockeye spawning stocks (names enclosed in box) and major sockeye salmon fisheries (box with name outside) on the south coast.

Although the Fraser River sockeye stocks dominate the catch in this region, six other Canadian sockeye stocks located in the Strait of Georgia and Johnstone Strait are included, as is a U.S. stock, spawning in the Cedar River flowing into Lake Washington (near Seattle).

The total Fraser River sockeye run is large and complex, consisting of 8–10 major stocks and 30–40 minor stocks. These stock groupings are described elsewhere (e.g. Killick 1955; Henry 1961) by the International Pacific Salmon Fisheries Commission (IPSFC), the regulatory agency set up by international treaty which had jurisdiction over these stocks prior to 1985. For the purposes of this analysis, the Fraser River stocks have been combined into two major components determined by timing: early and late. The early component consists of stocks which enter the mouth of the river from late June to late August (without delays), while the late component typically delays off the mouth of the river 3–6 wk and then enters between early September and mid-October. The early component in 1982 is small and consists primarily of fish returning to the Chilko, Stellako, and Birkenhead rivers. The late component in 1982 is numerically dominant and is almost entirely made up of fish returning to the Adams and Shuswap rivers in central British Columbia.

Description of data sources and data preparation steps

The input data into the reconstruction analysis consist of four categories: (1) total escapement by stocks; (2) proportional timing of escapement by time period; (3) catch by fishery by time period; and (4) order of migration through the fishery for each stock, and time resident in each fishery. A great variety of data is required and some preparation is needed before input. The following is a description of the data used in the 1982 reconstruction of the southern British Columbia/Puget Sound sockeye run.

Escapement — Escapement estimates vary in quality depending on the agency involved. For the single U.S. stock (Cedar River), escapement counts are made at a lock at the outlet of the canal draining Lake Washington in Seattle. These daily counts are then compared with float counts and historical tower counts made in the spawning river. There is also a significant freshwater fishery in Lake Washington on these stocks.

TABLE 3. Half-weekly escapements by stock for 1982 southern British Columbia and Puget Sound sockeye. See text for details of calculation. The split between southern and northern approaches is based on IPSFC estimates for the total annual diversion and is applied at escapement. The Nimpkish "protected" stock is not passed through the major gauntlet fishery in Area 12 because of management action to protect this stock. The proportion of the stock which is "protected" is estimated by local managers for the weeks during which boundary changes were in place.

Time period	Nimpkish	Nimpkish protected	Fulmore	Heydon	Phillips	Sakinaw	Early Fraser south	Early Fraser north	Late Fraser south	Late Fraser north	Lake Washington
MY-4A	0	0	0	0	0	0	0	0	0	0	0
MY-4B	0	0	0	0	0	0	0	0	0	0	0
JN-1A	0	0	0	0	0	0	0	0	0	0	0
JN-1B	0	0	0	0	0	0	0	0	0	0	0
JN-2A	0	0	0	0	0	0	0	0	0	0	0
JN-2B	994	0	0	0	0	0	0	0	0	0	0
JN-3A	994	0	0	0	0	0	0	0	0	0	0
JN-3B	1 087	0	2	0	1	0	0	0	0	0	39
JN-4A	1 087	0	8	1	10	0	0	0	0	0	509
JN-4B	4 756	0	27	5	52	4	0	0	0	0	3 053
JL-1A	4 756	0	71	18	182	18	0	0	0	0	11 195
JL-1B	7 644	0	142	47	472	62	4 091	722	0	0	27 988
JL-2A	1 529	6 115	223	94	944	161	7 225	1 275	0	0	50 378
JL-2B	1 160	4 639	278	148	1 484	321	3 730	658	0	0	67 170
JL-3A	1 160	4 639	278	185	1 855	504	16 190	2 857	0	0	67 170
JL-3B	1 212	4 847	223	185	1 855	631	19 319	3 409	0	0	50 378
JL-4A	1 212	4 847	142	148	1 484	631	7 245	1 278	0	0	27 988
JL-4B	648	2 591	71	94	944	504	13 860	2 446	0	0	11 195
JL-5A	648	2 591	27	47	472	321	20 679	3 649	0	0	3 053
JL-5B	52	208	8	18	182	161	10 664	1 882	0	0	509
AU-1A	259	0	2	5	52	62	30 836	5 442	0	0	39
AU-1B	163	0	0	1	10	18	40 587	7 162	0	0	0
AU-2A	163	0	0	0	1	4	264 320	46 645	0	0	0
AU-2B	0	0	0	0	0	0	36 224	6 392	0	0	0
AU-3A	0	0	0	0	0	0	29 943	5 284	0	0	0
AU-3B	0	0	0	0	0	0	25 904	4 571	0	0	0
AU-4A	0	0	0	0	0	0	60 616	10 697	20	7	0
AU-4B	0	0	0	0	0	0	82 188	14 504	338	119	0
SP-1A	0	0	0	0	0	0	72 143	12 731	2 708	954	0
SP-1B	0	0	0	0	0	0	16 397	2 894	13 538	4 768	0
SP-2A	0	0	0	0	0	0	0	0	47 383	16 689	0
SP-2B	0	0	0	0	0	0	0	0	123 197	43 393	0
SP-3A	0	0	0	0	0	0	0	0	246 393	86 785	0
SP-3B	0	0	0	0	0	0	0	0	387 189	136 377	0
SP-4A	0	0	0	0	0	0	0	0	483 986	170 471	0
SP-4B	0	0	0	0	0	0	0	0	483 986	170 471	0
OC-1A	0	0	0	0	0	0	0	0	387 189	136 377	0
OC-1B	0	0	0	0	0	0	0	0	246 393	86 785	0
OC-2A	0	0	0	0	0	0	0	0	123 197	43 393	0
OC-2B	0	0	0	0	0	0	0	0	47 383	16 689	0
OC-3A	0	0	0	0	0	0	0	0	13 538	4 768	0
OC-3B	0	0	0	0	0	0	0	0	2 708	954	0
OC-4A	0	0	0	0	0	0	0	0	338	119	0
OC-4B	0	0	0	0	0	0	0	0	20	7	0
OC-5A	0	0	0	0	0	0	0	0	0	0	0
OC-5B	0	0	0	0	0	0	0	0	0	0	0
Total	29 524	30 477	1 502	996	10 000	3 402	762 161	134 498	2 609 504	919 126	320 664
Grand total											4 821 854

Although daily escapement timing is available for this stock, only the median and the duration were used to which a binomial timing curve was fitted. All terminal catches for this stock, both Indian and all-citizen, freshwater and marine, have been added into the escapement and distributed according to the binomial escapement timing curve.

Escapement estimates for individual Fraser River stocks were obtained from IPSFC annual reports (largely based on mark–recovery experiments) and were summed over stocks to make the appropriate composite stock escapement. Escapement timing proportions were taken from indices of abundance calculated from test fishing catches from the lower part of the Fraser River. The total Fraser River sockeye run is divided into an early and late component. That divison is made separately each year when test fishing in the Fraser River drops to a low point before increasing during the "late" run. This low point in the

test fishing forms an arbitrary division between early and late run. Such a sharp distinction between the runs is probably unrealistic, but detailed racial composition of the test catch is not available. This method of dividing the escapement timing also leads to unreasonable compression of the late Fraser stocks. This is because the late Fraser stocks delay at least 4 wk off the mouth of the Fraser before entering the river. Entry into the river appears to cover a shorter time span than entry of fish into the delaying area. If the compressed timing curve is used in the escapement, then very high harvest rates are estimated in the fisheries keying on the late run stocks, and a badly skewed entry timing curve is estimated (30–50% of the run is estimated to enter in the first week or so). To get around this problem, the test fishery provides a peak timing and an extended binomial distribution is fitted to the late Fraser stock escapement timing curve.

The escapements for the other Canadian stocks are estimated visually by Department of Fisheries and Oceans (DFO) personnel. These estimates vary in quality but are generally felt to be poor and be too low. Test fishing catches in upper Johnstone Strait are routinely separated between Fraser and non-Fraser stocks based on the analysis of scales. From this, a non-Fraser test fishing index of abundance is calculated which forms the basis of the escapement timing curve of the Nimpkish stock (backdated one half week from the test fishery). The other non-Fraser stocks are fitted with binomial escapement timing curves, with their peaks set relative to the Nimpkish peak and the breadth the same as the Nimpkish run. The escapements by stock and time period are presented in Table 3.

Catch — All catches from waters outside of IPSFC management control come from DFO and Washington Department of Fisheries (WDF) catch statistics. These are summarized from fish sales transactions between fishermen and processors and are reported weekly, although available on a daily basis. Within IPSFC convention waters, catch statistics are available on a daily basis. In Canadian waters, they are generally obtained through direct contact with processors. In U.S. waters, the IPSFC gathers a daily copy of each fisherman's transaction records. All daily catches are allocated to half-weeks by the following convention: (a) first half week is composed of catches from Sunday to Wednesday; (b) second half week is made up of catches from Thursday to Saturday.

The following is a description of each fishery used in the reconstruction (see Fig. 1 for locations of all fisheries):

(1) Area 11: This is a minor gillnet and troll fishery located at the northern end of Vancouver Island in Queen Charlotte Sound. The troll catch is back-dated one full week to allow for reporting lags and is then split evenly between half weeks. The gillnet catch, which is always very small, is added into the Area 12 net catch with no lags.

(2) Areas 12 and 13: This is the major northern approach fishery in Johnstone Strait on sockeye stocks and is primarily a seine fishery, although gillnet and troll gear also operate in this area. Because of the length of Johnstone Strait (over 200 km), it is divided into an upper and lower half (i.e. Area 12 = upper, Area 13 = lower) because sockeye stocks take a full week to migrate through this fishery. All weekly catch is allocated entirely to the first half week of each week, including the troll catch. This is because all gears (in 1982) had the same fishing times, generally from Sunday night to Tuesday night of each week.

(3) Areas 14–16: This is a minor fishery in Georgia Strait. Almost all catch is by a seine fishery located in a small channel on the southeast tip of Texada Island. There is also a small troll fishery in this area.

(4) Nonconvention troll: There is an extensive troll fishery for sockeye off the west coast of Vancouver Island. This fishery is somewhat artificially divided by the administrative boundaries of IPSFC management control (at the 49th parallel). In general, this fishery is a large-boat fishery north of Estevan Point (DFO Statistical Areas 25–27). South of this point, there is a predominance of smaller trollers delivering to processors every day. Only part of this fishery (northern half of DFO Statistical Area 24) is in this designation. Allocation of the weekly catch in this fishery was done in the following manner: (a) the entire west coast of Vancouver Island catch for 1982 was summed (DFO Statistical Areas 21–27); (b) the total convention troll catch as estimated by the IPSFC south of the 49th parallel was then subtracted to give the total annual catch north of the 49th parallel; (c) the weekly proportion of catch was estimated by moving the weekly catches in Areas 25–27 ahead 1 wk and adding half of the Area 24 catch for that week and then dividing by the total catch from June to October; (d) the proportion was then multiplied by (b) above and the result divided equally between each half of the week.

(5) Convention troll: This is the balance of the west coast of Vancouver Island troll fishery and is largely a day-boat fishery. The catch in this fishery is estimated daily from deliveries to processing plants.

(6) Juan de Fuca Strait (DFO Area 20): This is a seine and gillnet fishery on both sides of the international boundary, although the catch taken in the U.S. waters is less than 10% of that in Canadian waters. The two fisheries are summed in this analysis.

(7) U.S. Net: This is a complex fishery using seine, gillnet, and reef net gear. It has two main centres: one off the south end of San Juan Island (Salmon Banks) and the other in Point Roberts just south of the mouth of the Fraser. There are also several other areas of less importance, including the west coast of Whidbey Island (West Beach). All these fisheries are combined into a single fishery, since it takes about one half week for a sockeye to traverse the entire fishery.

(8) District No. 1: This is the fishery in the general area of the mouth of the Fraser River. It is largely a gillnet fishery which operates in the main channel of the river up to 80 km from the mouth. Later in the season (usually in September), the fishery moves out of the channel into the shallow waters of the estuary. There is also a small troll fishery off the mouth of the Fraser as well as the occasional seine fishery off the islands on the east coast of Vancouver Island (not in 1982).

The catches by fishing area and time period are presented in Table 4.

Migration order and approach — The path of migration by each sockeye stock is derived from previously published information (Henry 1961; Killick 1955) and from interviews with appropriate management staff (J. Ames, WDF; J. Woody, PSC; A. Gould, DFO). In general, sockeye can approach the Fraser River either from the north through Johnstone Strait or from the south through Juan de Fuca Strait. Because the predominant route of entry has been through Juan de Fuca Strait, the proportion of fish using the northern route is referred to as the "diversion rate." In this analysis, it is assumed that the Lake Washington stock approaches exclusively from the south and that the Canadian stocks from Johnstone Strait/Strait of Georgia approach exclusively from the north.

TABLE 4. Half-weekly catches of sockeye in southern British Columbia and Puget Sound sockeye. See text for details of fishery locations and assignment of catch to time periods.

Week	Area 11	Area 12	Area 13	Areas 14–16	Nonconvention troll	Convention troll	Area 20	U.S. Net	District 1
MY-4A	0	0	0	0		0		0	0
MY-4B	0	0	0	0	0	0		0	0
JN-1A	0	0	0	0	0	0	0	0	0
JN-1B	0	0	0	0	0	0	0	0	0
JN-2A	0	0	0	0	0	0	0	0	0
JN-2B	0	0	0	0	0	0	0	0	0
JN-3A	0	0	0	0	0	0	0	0	0
JN-3B	0	0	0	0	2	0	0	0	0
JN-4A	4	177	0	0	246	0	0	0	0
JN-4B	4	0	0	0	246	0	0	0	7
JL-1A	28	732	2	0	1 120	7	0	0	5
JL-1B	28	0	0	0	1 120	97	49	0	33
JL-2A	238	12 376	8 427	71	3 572	138	297	0	157
JL-2B	238	0	0	0	3 572	104	640	0	125
JL-3A	165	21 192	3 280	0	4 989	384	421	0	162
JL-3B	165	0	0	0	4 989	1 302	332	0	172
JL-4A	219	30 575	10 175	1 150	50 207	5 224	834	298	233
JL-4B	219	0	0	0	50 207	12 827	641	782	298
JL-5A	560	106 785	31 807	917	107 795	84 849	2 335	157 966	23 577
JL-5B	560	0	0	0	107 795	148 899	2 207	840	1 722
AU-1A	3 805	29 247	5 620	1 190	239 499	60 315	4 168	453 065	21 272
AU-1B	3 805	0	0	0	239 499	45 396	10 551	1 063	11 982
AU-2A	4 117	409 604	144 132	11 666	231 692	94 598	18 459	379 771	156 747
AU-2B	4 117	0	0	0	231 692	97 122	1 785	74 118	4 486
AU-3A	1 046	451 679	195 237	55 230	70 343	96 421	431 423	526 181	101 747
AU-3B	1 046	0	0	0	70 343	56 322	7 573	155 275	32 576
AU-4A	12	123 895	40 189	53 090	11 975	16 441	575 947	500 337	60 119
AU-4B	12	0	0	0	11 975	3 663	12 152	30 540	2 371
SP-1A	9	11 961	19 164	5 809	1 975	2 768	698 043	462 833	11 304
SP-1B	9	0	0	0	1 975	879	5 626	26 825	330
SP-2A	19	3 444	3 708	224	2 246	393	1 759	2 627	41 241
SP-2B	19	0	0	0	2 246	5	315	33	100
SP-3A	0	2 953	2 584	0	4 342	1	1 774	136	100
SP-3B	0	0	0	0	4 342	0	8	109	94
SP-4A	0	2	261	146	568	0	11	15	5 092
SP-4B	0	0	0	0	568	0	7	33	165 985
OC-1A	0	151	107	15	96	0	5	313	156 383
OC-1B	0	0	0	0	96	0	0	78	342
OC-2A	0	28	26	0	0	0	0	4	63 391
OC-2B	0	0	0	0	0	0	0	2	60
OC-3A	0	69	15	0	0	0	0	1	39
OC-3B	0	0	0	0	0	0	0	136	23
OC-4A	0	68	631	0	0	0	0	40	34
OC-4B	0	0	0	0	0	0	0	1	7
OC-5A	0	0	0	0	0	0	0	1	19
OC-5B	0	0	0	0	0	0	0	2	10
Total	20 444	1 204 938	465 365	129 508	1 461 334	728 255	1 777 362	2 773 425	862 345
Grand total									9 422 976

The proportion of Fraser stocks approaching from the north was set at the rate estimated by the IPSFC (1982 Annual Report). This was done because no other information is available that suggests another value be used. The migration path followed by each stock is presented in Table 5.

Results

The total run for each sockeye stock ranged from 2200 to 10.5 million, while the total exploitation rate on each stock ranged from 3 to 72% (Table 6). The total Fraser sockeye run is calculated at 13.8 million. The estimate published by the IPSFC for the total Fraser sockeye run is 13.9 million (1982 Annual Report) and this includes catch north of Cape Caution which was not considered in this analysis. The proportional split between early and late components was calculated at 23–77%. Using racial scale analysis on direct samples taken from each major fishery, IPSFC split the early and late components at 22–78% (IPSFC, unpubl. data). This stock split is quite sensitive to the estimate of the proportion entering from the north. The estimate published by the IPSFC (22%, 1982 Annual Report) is for the total Fraser run at the upper end of Vancouver Island. The reconstruction analysis splits the approach route at escapement. The reconstruction estimate of the diversion rate is also 22% of the total Fraser River run.

There are no independent estimates of the total run for the other Canadian stocks. The Lake Washington stock, however,

TABLE 5. Migration order by fishery for each stock in the 1982 southern sockeye reconstruction. Fisheries are abbreviated and correspond to the catches listed in Table 4. Every fish in a particular stock is assumed to pass from fishery to indicated fishery whenever a time period is incremented.

Time period	Nimpkish	Nimpkish protected	Fulmore	Heydon	Phillips	Sakinaw	Early Fraser south	Early Fraser North	Late Fraser south	Late Fraser north	Lake Washington
1	Area 11	Area 11	Area 11	Area 11	Area 11	Area 11	NCVNT	Area 11	NCVNT	Area 11	NCVNT
2	Area 12	Hold	Area 12	Area 12	Area 11	Area 12	CVNT	Area 12	CVNT	Area 12	CVNT
3	Hold	Hold	Hold	Area 13	Area 13	Area 13	Area 20	Area 13	Area 20	Area 13	Area 20
4				Hold	Hold	Areas 14–16	U.S. Net	Areas 14–16	U.S. Net	Areas 14–16	Hold
5						Hold	Dist. 1	Dist. 1	Hold	Hold	
6									Hold	Hold	
7									Hold	Hold	
8									Hold	Hold	
9									Hold	Hold	
10									Hold	Hold	
11									Hold	Hold	
12									Hold	Hold	
13									Dist. 1	Dist. 1	

TABLE 6. Summary of total run, total catch, and realized exploitation rate (total catch divided by total run) for each stock in the weekly sockeye reconstruction in 1982.

Stock	Total run	Total catch	Total escapement	Percent exploitation
Nimpkish	38 723	9 199	29 524	23.8
Nimpkish protected	30 639	162	30 477	0.5
Total	69 362	9 362	60 001	13.5
Fulmore	2 671	1 169	1 502	43.8
Heydon	2 221	1 225	996	55.2
Phillips	22 300	12 300	10 000	55.2
Sakinaw	7 989	4 587	3 402	57.4
Early Fraser south	2 759 480	1 997 319	762 161	72.4
Early Fraser north	477 943	343 445	134 498	71.9
Total	3 237 423	2 340 764	896 659	72.3
Late Fraser south	8 014 785	5 405 281	2 609 504	67.4
Late Fraser north	2 540 655	1 621 529	919 126	63.8
Total	10 555 440	7 026 810	3 528 630	66.6
Total Fraser	13 792 863	9 367 574	4 425 289	67.9
Lake Washington	329 991	9 327	320 664	2.8

was estimated by WDF in 1983 at 324 000 as compared with 330 000 by reconstruction.

Comparison of catch by stock in each area also shows correspondence with the racial scale data. The total contribution by Fraser stocks to the Johnstone Strait fishery is estimated by the reconstruction at 1 787 000 (Table 7) as compared with the IPSFC estimate of 1 811 000 (1982 Annual Report). The catch in nonconvention waters off the west coast of Vancouver Island is calculated at 1 443 000 by the reconstruction. The comparable IPSFC estimate is 1 599 000 but this includes at least 100 000 caught off the west coast of the Queen Charlotte Islands and in Millbanke Sound (central British Columbia). The Lake Washington contribution to the British Columbia seine and troll fishery in IPSFC convention waters was estimated by the reconstruction at 3000 and by WDF at 2950.

Another useful output of the reconstruction is the estimate of harvest rate within each time period for each fishery. Racial analysis does not provide this. The weekly harvest rates as estimated from reconstruction vary from near 20 to 80% (Fig. 2). We have excluded harvest rates in the troll fishery and those near the end of the timing distributions.

A further refinement of the reconstruction method would be to explore the relationship between harvesting effort and the resultant half-weekly harvest rate. Before proceeding with such an analysis, different types of fishing gear (seine net, gillnet) should be calibrated and combined into a total measure of effort. This was done by adjusting the total effort of a gear type by its catch-per-unit-effort (CPUE) relative to the CPUE of seine gear (the most efficient gear). Therefore, half-weekly harvest rate is plotted against total seine boat day equivalents (Fig. 3). These plots show that for all four Canadian fisheries examined, there is very little change in the realized harvest rate, even though effort varies over 2.4 times in Area 20 and 12.8 times in Area 13. The scatter plots show great variation in harvest rate, but

TABLE 7. Summary of total annual catch for each stock within each fishing area considered in the 1982 weekly sockeye reconstruction.

Stock	Area 11	Area 12	Area 13	Areas 14–16	Nonconvention troll	Convention troll	Area 20	U.S. Net	District 1
Nimpkish	83	9 116	0	0	0	0	0	0	0
Nimpkish protected	162	0	0	0		0	0	0	0
Total	245	9 116	0	0	0	0	0	0	0
Fulmore	10	1 159	0	0	0	0	0	0	0
Heydon	10	769	446	0	0	0	0	0	0
Phillips	97	7 724	4 479	0	0	0	0	0	0
Sakinaw	36	2 914	1 524	111	0	0	0	0	0
Early Fraser south	0	0	0	0	337 717	234 839	289 223	775 825	359 714
Early Fraser north	2 591	185 636	79 991	11 751	0	0	0	0	63 477
Total	2 591	185 636	79 991	11 751	337 717	234 839	289 223	775 825	423 191
Late Fraser south	0	0	0	0	1 105 002	492 879	1 485 694	1 996 974	324 733
Late Fraser north	17 436	994 344	377 885	117 486	0	0	0	0	114 378
Total	17 436	994 344	377 885	117 486	1 105 002	492 879	1 485 694	1 996 974	439 111
Total Fraser	20 027	1 179 980	457 876	129 237	1 442 719	727 718	1 774 917	2 772 799	862 302
Lake Washington	0	0	0	0	6 357	536	2 433	0	0

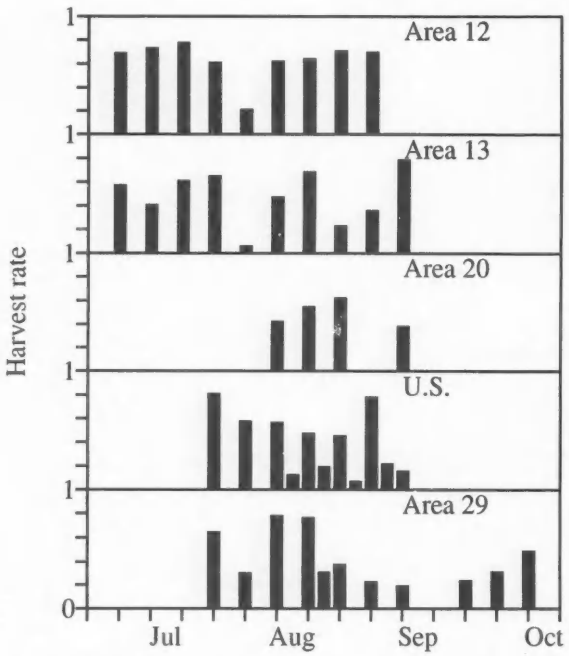

FIG. 2. Half-weekly harvest rates by major fishing area in the 1982 sockeye reconstruction. Half-weeks of minor catches near the beginning and end of the year were omitted because the model indicated 100% harvest.

with no systematic trend due to effort. This agrees with the general understanding of these fisheries: there are a limited number of good fishing locations and large number of boats simply causes the fishermen to queue up for these sites. Areas 12, 13, and 29 all show a few points which appear to be outliers (Fig. 3, circled points). In each case, these points correspond to boundary reductions which affect the realized harvest rate by reducing the number of available fishing locations.

The major exception to the above trend is the fishery designated U.S. Net in the reconstruction (Fig. 3). This is a large, complex fishery composed of several fishing areas. In this case, there appears to be a trend of increasing harvest rate with increasing effort. Again, this corresponds to the intuition of managers, who characterize this fishery as containing many paths of approach and many suitable fishing locations. Long queues for favored fishing locations are rarely seen in this fishery.

The reconstruction analysis also gives estimates of timing of the entering run by stock. Peak entering week ranged from late June (Nimpkish, Lake Washington) to early August (late Fraser) (see Fig. 4). The early Fraser component was later than all other stocks except the late Fraser.

Annual Reconstruction of the 1982 Sockeye Run

The same data used in the half-weekly reconstruction of the 1982 run were analyzed on an annual basis using the procedure described previously. In general, the annual method tends to overestimate the size of the late Fraser runs while underestimating the early Fraser component (relative to the half-weekly reconstruction, Table 8). This happens because of the assumption of equal vulnerability; each stock appearing in the same

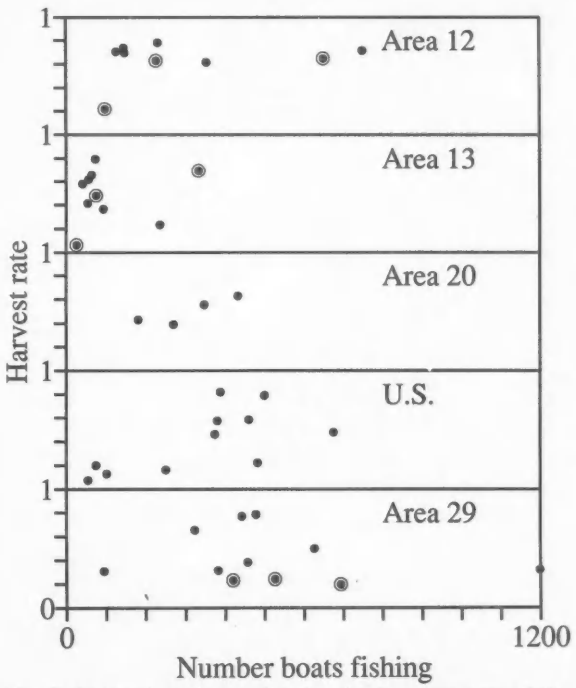

FIG. 3. Scatter plots of total effort in seine boat days against half-weekly harvest rates estimated by the reconstruction of southern sockeye in 1982. Gillnet boat days were converted to seine-net boat days by the ratio of the relative CPUE of each gear type in each fishery and half-week. Circled points for Areas 12 and 13 and District 1 (Area 29) indicated reduced fishing area. Boat days for Area 29 are gillnet only and the estimated harvest rates are daily instead of half-weekly.

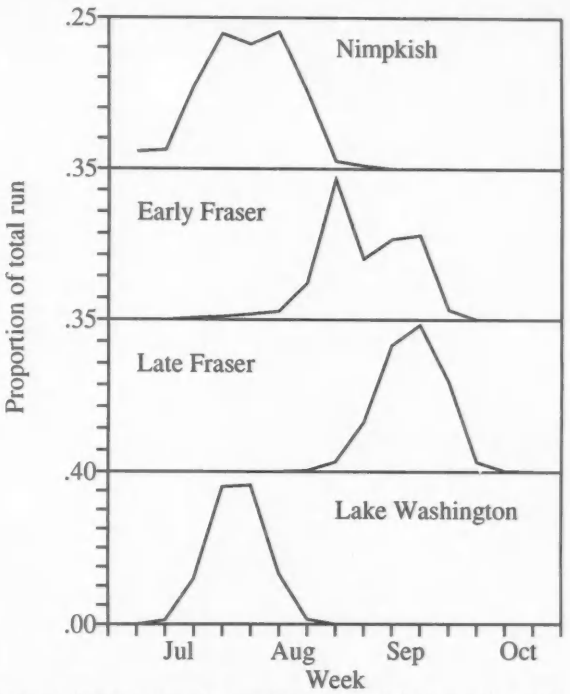

FIG. 4. Weekly proportion entering for each of the four major stocks in the 1982 southern sockeye reconstruction. Fraser River curves (both early and late stocks) are summed (weighted) from both approaches and hence represent the timing at the north end of Vancouver Island.

fishery will have an equal harvest rate applied to it. On an annual basis, this means that stocks which appear at different times in a season will still have the same harvest rate applied. This is obviously not true for situations where the fishing effort is distributed unequally through the season. For instance, the Lake Washington run is assigned larger catches from the Area 20 net fishery and the west coast of Vancouver Island troll fisheries (Table 9). This is very unlikely because those fisheries did not have very high exploitation rates when the majority of the Lake Washington run was passing by. The weekly method of reconstruction is essentially a weighting procedure that corrects for seasonal changes in exploitation rates in different fisheries.

It is interesting to note that the overall annual harvest rates estimated by both the weekly and annual reconstruction procedures are the same. This is a result of the definiton of overall annual harvest rate being the total catch divided by total catch plus escapement.

General Assumptions and Problems

While it is clear that reconstruction can estimate stock composition and harvest rates not normally available by other methods, reconstruction makes many assumptions which are often unpalatable to fishery managers. The major assumptions are (1) all fish of the same "clump" move together through the fishery and (2) equal vulnerability to fishing gear by all stocks. Reconstruction can only be used on fish migrating towards their natal streams; it is not an appropriate tool for juvenile or "feeder" fish.

Both of these assumptions can be overcome (see later section), but this requires further assumptions. In addition, salmon stocks may chose several approach routes to their natal streams, and none of the migration paths is necessarily the shortest route to their natal stream. The reconstruction procedure assumes we can estimate how many of the fish came via each possible approach path. We have assumed that the proportion choosing each approval path was constant throughout the year — this is an assumption of convenience. In general, the reconstruction method allows the manager to estimate stock contributions to a fishery and harvest rates while holding the proportion of the stock choosing a particular migration path constant.

Sensitivity of Reconstruction to Poor Data

There are four basic types of data error which will affect the results of the reconstruction analysis: (1) errors in escapement counts, (2) errors in escapement timing, (3) errors in catch timing, and (4) errors in migration route and speed. All four types of error affect the analysis in different ways and all four are potentially quite serious.

Errors in escapement count are of primary concern if some stocks are underestimated relative to the others. A constant bias in escapement counts will not affect stock contribution estimates, only the harvest rate estimates. If some stocks are systematically underestimated in the escapement, then too few fish from the catch will be allocated to these stocks and their stock contributions will be underestimated.

TABLE 8. Summary of total run, total catch, and exploitation rate for each stock in the annual sockeye reconstruction for 1982.

Stock	Total run	Total catch	Total escapement	Percent exploitation
Nimpkish	48 570	19 046	29 524	39.2
Nimpkish protected	30 676	199	30 477	0.7
Total	79 245	19 244	60 001	24.3
Fulmore	2 471	969	1 502	39.2
Heydon	2 181	1 186	996	54.4
Phillips	21 908	11 908	10 000	54.4
Sakinaw	8 218	4 816	3 402	58.6
Early Fraser south	388 201	253 703	134 498	65.4
Early Fraser north	2 393 966	1 631 805	762 161	68.2
Total	2 782 167	1 885 508	896 659	67.8
Late Fraser south	2 652 871	1 733 745	919 126	65.4
Late Fraser north	8 196 513	5 587 009	2 609 504	68.2
Total	10 849 385	7 320 755	3 528 630	67.5
Total Fraser	13 631 551	9 206 262	4 425 289	67.5
Lake Washington	499 254	178 590	320 664	35.8

TABLE 9. Summary of total catch for each stock within each fishing area considered in the 1982 annual sockeye reconstruction.

Stock	Area 11	Area 12	Area 13	Areas 14–16	Nonconvention troll	Convention troll	Area 20	U.S. Net	District 1
Nimpkish	315	18 731	0	0	0	0	0	0	
Nimpkish protected	199	0	0	0	0	0	0	0	
Total	514	18 731	0	0	0	0	0	0	
Fulmore	16	953	0	0	0	0	0	0	0
Heydon	14	842	330	0	0	0	0	0	0
Phillips	142	8 449	3 317	0	0	0	0	0	0
Sakinaw	53	3 169	1 244	349	0	0	0	0	0
Early Fraser south	0	0	0	0	315 461	157 210	383 683	626 930	148 520
Early Fraser north	2 515	149 710	58 781	16 487	0	0	0	0	26 209
Total	2 515	149 710	58 781	16 487	315 461	157 210	383 683	626 930	174 729
Late Fraser south	0	0	0	0	1 080 084	382 591	1 313 663	2 146 495	508 508
Late Fraser north	17 190	1 023 084	401 692	112 671	0	0	0	0	179 108
Total	17 190	1 023 084	401 692	112 671	1 080 084	382 591	1 313 663	2 146 495	687 616
Total Fraser	19 705	1 172 794	460 473	129 158	1 395 545	697 346	1 697 346	2 773 425	862 345
Lake Washington	0	0	0	0	65 789	80 016	80 016	0	0

Errors in the escapement timing estimates are potentially very serious and are difficult to detect or correct. If too few (or too many) fish from a particular stock are assigned to a specific time block, then the allocation of catch throughout all succeeding time blocks for this clump of fish will be incorrect. This could lead to very high or low estimates of weekly harvest rates as well as skewed entrance timing curves. Typically, weeks with high harvest rates will appear or the entrance timing curve will look very unusual. This is because large amounts of catch are added into relatively small escapement clumps. This problem can be solved by increasing the breadth of the timing curve (i.e. increasing the number of fish in the tails of the curve).

Errors in catch timing are more straight forward. Misallocation of catch by time period and area is common in most fisheries. This problem is partially solved by pooling time periods and areas. The use of daily catch data is prone to this type of error and we do not recommend this fine a time scale.

Errors in migration routing are almost impossible to detect. These fall into two categories: errors in the allocation of stock to an approach route and errors in the timing of passage between fisheries. In the first case, when stocks must be allocated to a particular approach by means of a static proportion (e.g. the "diversion rate"), the best that can be hoped for is to bracket the range of possible proportions. Unless there exists some

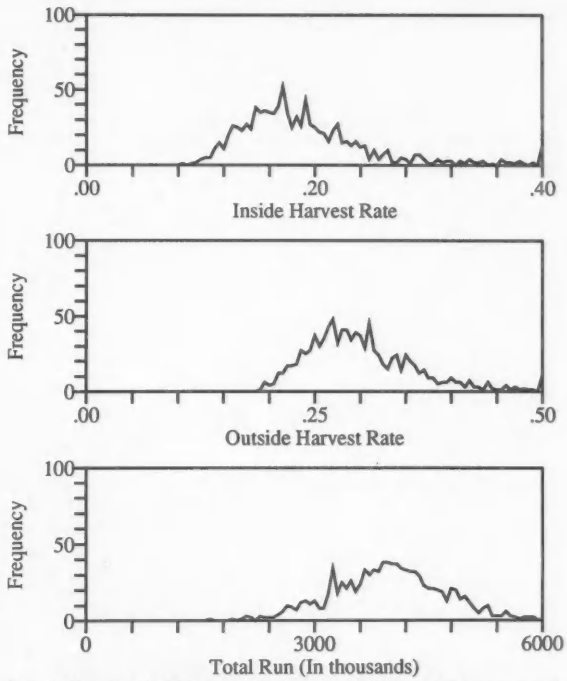

FIG. 5. Results of 1000 Monte Carlo runs of 1978 pink salmon annual reconstruction in British Columbia Statistical Area 8. Parameters used are discussed in the text.

external measurement (e.g. a harvest rate) to scale the analysis, the only internal measure is the overall harvest rate and this provides little information to judge the reliability of migration path assumptions.

Errors in the timing of passage between fisheries are probably less serious. The migration passage times we used are averages based on tagging studies and there are undoubtedly some fish lagging behind and some moving faster than average. It is unknown how much these differential movement rates affect the results of reconstruction, but one could develop a stochastic movement version of reconstruction.

Potential Refinements in Reconstruction Methods

The reconstruction methods we have described are currently in use but are not necessarily as biologically realistic as we might wish. In this section we describe three refinements in reconstruction methods that could be implemented, but we have not done so at present. These three refinements are (1) differential vulnerabilities of stocks, (2) calculation of confidence limits of reconstruction outputs, and (3) the inclusion of non-catch fishing mortality.

Differential Vulnerability

Suppose we have data that give us the relative vulnerability V_{ij} of each stock (i) in each area (j). V would normally be 1, but less vulnerable stocks would have $V < 1$ and any stock that was unusually vulnerable would have $V > 1$. The relationship between catch, V, and abundance is now

$$(6) \quad C_{ijkl} = h_{jk} V_{ij} N_{ijkl}.$$

With uniform vulnerability ($V = 1$) the harvest rate is calculated as

$$(7) \quad h_{jk} = \frac{C_{jk}}{C_{jk} + \sum_i \sum_l P_{ijkl}},$$

that is, the denominator is the catch in the area and time plus the number of fish left after the fishery summed over all possible stocks and clumps. The number present before the fishery is

$$(8) \quad N_{ijkl} = \frac{P_{ijkl}}{1 - h_{jk}}.$$

When there is differential vulnerability the relationship for harvest rate is

$$(9) \quad h_{jk} = \frac{C_{jk}}{\sum_i \sum_l \frac{P_{ijkl}}{(1 - h_{jk} V_{ij})} V_{ij}}.$$

This equation must be solved iteratively for the h's. Once the h's are known, then the N's can be solved from

$$(10) \quad N_{ijkl} = \frac{P_{ijkl}}{1 - h_{jk} V_{ij}}.$$

Adding these changes to the computer program which does the reconstruction would be very simple. However, we have not yet encountered any data on differential vulnerability that would be adequate to use in a reconstruction.

Determination of Confidence Limits

It would, of course, be reassuring to have confidence in the specific outputs from the reconstruction given the uncertainties in the inputs. With Monte Carlo methods we can specify a mean and a variance (or any probability distribution) for each data input, run the reconstruction a few hundred times, and then examine the distribution of the output parameters. We can describe each output parameter (stock contributions, harvest rates) by a mean and a variance.

Figure 5 shows the distribution of estimated harvest rates and total run estimates for the annual pink salmon reconstruction given in Table 1. We assumed that the coefficient of variation of the escapement estimates was 0.3 and the coefficient of variation of the catch estimates was 0.1. We then performed 1000 Monte Carlo trials drawing catch and escapement estimates from the probability distributions specified by the mean estimates from Table 1 and a standard deviation calculated using the coefficients of variation described above.

The simplicity of this method hides some practical problems with each type of data. While we might be confident about the catch data, we might recognize uncertainty and specify a variance for the catch in each space and time. This would assume an uncorrelated error between different locations and times. An alternative view of uncertainty is that we might believe that the reported catch constitutes some proportion of the total catch and that we are uncertain about that proportion: it might be 0.8, 0.9, or 0.99. We might then assume that the only uncertain parameter is the proportion of catch reported and inflate the numbers in all fisheries by a proportion drawn from a probability distribution.

As with catch, we might believe that there is no uncertainty in the escapement data, or assign specific uncertainties to each

escapement value. It is more likely that for each stock, we would want to consider uncertainty about the total escapement, the timing of the peak of the escapement, and variability in the time of arrival.

Migration timing is more difficult to deal with because it takes only integer values. The best approach we can think of is to define the migration order as the sequence of areas the stock passes through and the number of time intervals the stock is resident in each area. Uncertainty about the number of intervals of residence in each area could be incorporated, and for each Monte Carlo run a different migration order could be generated. This requires that there typically be several time intervals of residence in each area so that either the time intervals must be small or the areas large.

It is difficult to deal with uncertainty about migration route in a stochastic fashion. Our only suggestion is that the heuristic methods of trying different orders we described earlier be used.

Noncatch Mortality

In some fisheries, there may be significant mortality of fish that is not reported as catch. Sources of noncatch mortality could be (1) gear induced mortality such as drop outs of dead fish from gill nets, (2) marine mammal kill and damage of fish that have been caught in nets, but not yet landed, and (3) nonreported catch. Inclusion of any of these factors is simple on a fishery by fishery basis. If one can estimate the percent of fish killed that are reported as catch, the "catch" estimate in that fishery is simply inflated by the appropriate percentage.

Preseason Planning using the Reconstruction Framework

The multi-time-period reconstruction framework uses catch by area and time, escapement by stock and time, and migration path and timing to calculate harvest rate by fishery, abundance by stock, and the timing of entry into the first fishery by stock. Reconstruction is an evaluative tool to ask what has happened. The assumptions of the reconstruction can be turned completely around to generate a planning tool that enables us to ask "what if" questions about different stock sizes and harvest rates.

The preseason planning model uses preseason forecast of run size and timing, fishing plans for harvest rate by area and time, as well as migration path and timing to calculate catch by stock area and time and escapement by stock and time. The preseason planning model allows managers to test alternative harvest patterns to try to achieve escapement targets on different stocks, or to evaluate consequences of different run strengths.

The equations that are used in the preseason planning model are

$$
\begin{aligned}
C_{ijkl} &= N_{ijkl}\, h_{jk} \\
P_{ijkl} &= N_{ijkl} - C_{ijkl} \\
N_{ijkl} &= P_{ij'(k-1)l} \\
j &= O_{i,m} \\
j' &= O_{i,m-1}
\end{aligned} \qquad (11)
$$

$N_{iO_{1}kl}$ are input data.

This model is identical to one used by Royce et al. (1963) and Paulik and Greenough (1966). Similar models of salmon movement dynamics have been described or used in Argue (1970) and Palmer (1972); it is the simplest description of the movement pattern of salmon through a gauntlet fishery. The multi-time-period reconstruction method is built on the same assumptions and serves to calculate what the important data (run size, timing by stock, and harvest rate by fishery) are. Given the model of equation 11, the reconstruction methods of equation 5 can be proved trivially.

A current use of the forward planning model and its complementary reconstruction is to remanage a specific fishery to see if, given a run strength and timing, we could have achieved specific escapement or catch targets. As management becomes more scientific and explicitly complex, these tools become a key component in examining alternatives.

Discussion

Salmon managers and scientists need to know harvest rates and stock contributions to fisheries to achieve management objectives and learn about the dynamics of the fleet and the fish. There are two methods available to determine the harvest rates in a fishery; a known number of individuals can be tagged prior to the fishery and the proportion of tags recovered produces an estimate of harvest rate, or reconstruction as described in this paper can be performed. Tagging studies may provide a more reliable estimate of harvest rate but are plagued by the problems of tagging mortality, tag loss, and unrecorded recoveries. We cannot generalize about the relative reliability of a tagging study or run reconstruction. Moreover, tagging studies are very expensive and are not a practical tool for ongoing harvest rate determination in the numerous salmon fisheries that take place each year. There is really no alternative to reconstruction for most fisheries. Tagging studies should serve to help calibrate the migration paths and vulnerabilities used in the reconstruction methods.

Catch contribution to mixed fisheries has been estimated in three basic ways: (1) direct identification of individuals or assessment of stock frequencies including electrophoresis, scale analysis, and morphometrics, (2) reconstructions, and (3) using long-term average contribution derived from one of the other two methods. This last method, in which it is assumed that a constant proportion of fish caught in a fishery come from each stock, will tend to overestimate catch contribution when a stock is weak and underestimate its contribution when the stock is strong. This has the result of seriously biasing stock recruitment studies (Wong 1982; Hilborn and Starr 1984) so that the optimum harvest rate is overestimated and the optimum escapement is underestimated. Despite the fact that this method is still occasionally used, we will not discuss it further.

Estimation of catch contribution by direct identification of scales, parasites, or electrophoresis can be highly reliable and will probably provide a better estimate than will run reconstruction. The uncertainties about migration path and timing may make run reconstruction less reliable than direct identification, but the results shown previously in this paper indicate good agreement between the two methods. Reconstruction methods are much less expensive than direct identification, but the PSC does do intensive scale analyses every year in its major fisheries, so these methods do present, in some cases, a practical alternative to run reconstruction. Generally, we think that direct identification would be a calibration tool for the reconstruction methods rather than a comlete replacement.

While we view the methods of run reconstruction presented in this paper as a beginning to formal methods of evaluating gauntlet fisheries, reconstruction is a powerful tool now available for salmon managers. It is useful because (1) It is the only method currently available and affordable for many fisheries, (2) it gives the harvest rate and catch contribution numbers that

are needed, (3) it tells a manager exactly what data are needed and provides him with a framework for determining how uncertainty in any data item will affect the estimates of reconstruction, (4) it provides a framework in which a manager can test the logical consistency of his assumptions (sometimes managers make assumptions that are clearly inconsistent with each other once they are laid out in the reconstruction format), and (5) by explicitly stating a methodology for calculating harvest rates and catch contribution, it allows us to move from undocumented intuitive management to scientific methods that can be tested and improved.

A major impediment to scientific salmon management is the lack of documentation of methods. Reconstruction as described in this paper is a replicable, testable methodology that can be improved or discarded in the future as knowledge accumulates. The scientific method requires that techniques can be replicated by other scientists and evaluated. The traditional method where each manager has his own methods and intuition developed over many years of experience is not scientific because it cannot be repeated by later managers. The purpose of reconstruction and similar methodologies is not to replace the experience gained by practicing managers, but to capture that experience so that it is useable by future managers.

Acknowledgements

The development of run reconstruction methods is difficult to trace. The annual reconstruction method used by Washington State Department of Fisheries was developed independently of our work and we thank Jim Ames of Washington Department of Fisheries for his cooperation. Mike Staley and C. J. Walters were instrumental in developing the multi-time-period methods and we acknowledge a major debt to them for both ideas and comments on the implementation. A. Charles, M. Staley, S. Wright, and an anonymous reviewer provided very helpful comments on various versions of this paper.

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Orthophosphate Concentrations in Lake Water: Analysis of Rigler's Radiobioassay Method[1,2]

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Tarapchak, S. J., and L. R. Herche. 1988. Orthophosphate concentrations in lake water: analysis of Rigler's radiobioassay method. Can. J. Fish. Aquat. Sci. 45: 2230–2237.

Rigler's radiobioassay method is frequently used to estimate maximum possible orthophosphate (P) concentrations in natural waters. An evaluation of the method, based on simulated P uptake by hypothetical phytoplankton communities, reveals that the Rigler value is not a consistent estimator of true maximum possible P concentration. Analyses show that all members of that family of curves for which the difference between true and assumed (or estimated) values of P is below the minimum half-saturation constant of a component species will pass through the plot's origin. A new upper bound, termed $\mathcal{R}$ (Rigler), which is the sum of the true ambient P concentration and the lowest half-saturation constant of a component species, is identified as a consistently distinguishable bound on maximum possible P concentrations in lake water. The $\mathcal{R}$ curve cannot be distinguished in a lake water experiment because of the complex behavior of uptake curves in the unobservable substrate region. A theoretical procedure, based on comparing uptake parameters for lake water samples and multispecific hypothetical communities, offers potential for calculating upper and lower limits on $\mathcal{R}$ in P-limited lake water samples.

La méthode de dosage radiobiologique de Rigler s'emploie fréquemment dans l'estimation de la concentration d'orthophosphate (*P*) la plus élevée possible dans les eaux naturelles. On a évalué cette méthode en simulant l'absorption de P par des communautés de phytoplankton hypothétiques: la valeur de Rigler n'est pas un estimateur constant de la concentration réelle maximale possible de P et des analyses ont montré que toutes les courbes du groupe où la différence entre les valeurs de P réelles et supposées (ou estimées) est inférieure à la constante minimale de semi-saturation d'une espèce composante passent au point d'origine du tracé. Une nouvelle borne supérieure, $\mathcal{R}$ (Rigler), qui correspond à la somme des valeurs réelles de la concentration de P dans le milieu et de la valeur la plus faible de la constante de semi-saturation d'une espèce, s'est révélée être une limite toujours identifiable de la concentration de P la plus élevée possible dans les eaux lacustres. Il est impossible de tracer la courbe $\mathcal{R}$ dans le cas des eaux lacustres à cause de la complexité du processus d'absorption dans la partie inobservable du substrat. Une méthode théorique a du potential pour le calcul des bornes supérieures et inférieures de $\mathcal{R}$ dans des échantillons d'eau lacustre à teneur limitée en P : elle est basée sur la comparaison de paramètres d'absorption s'appliquant à des échantillons d'eau lacustre et à des communautés d'espèces multiples hypothétiques.

Received September 25, 1987
Accepted August 4, 1988
(J9433)

Reçu le 25 septembre 1987
Accepté le 4 août 1988

Progress in understanding phosphorus (P) dynamics in aquatic systems has been hampered because ambient true orthophosphate (P_a) concentrations cannot be accurately measured (Rigler 1973; Tarapchak and Nalewajko 1986). Colorimetric methods may severely overestimate P_a because large amounts of molybdate-reactive P are released by acid hydrolysis (Rigler 1968; Paerl and Downes 1978; Stainton 1980; Tarapchak et al. 1982) or compromised by excessive color development (Tarapchak 1983). Chromatographic techniques (e.g. gel filtration) are appealing because of their sensitivity, but measure radioisotopic activity rather than concentration in P_a fractions (Lean 1973; Stainton 1980). Enzymatic methods (Pettersson 1979) are highly specific for P_a but are insensitive to $P_a < 1.0$ μg $P \cdot L^{-1}$ (Rigler 1966, 1973; Vollenweider 1968; Levine and Schindler 1980).

Despite our technological inability to accurately measure P_a in P-limited systems, Rigler's (1966) radiobioassay method has provided an attractive alternative (Brown et al. 1978; Smith and Kalff 1982). According to Rigler, the bioassay estimates maximum possible orthophosphate concentrations (MPPC). Hence, the problem of precise P_a estimation apparently has been alleviated because at least an upper bound on P_a can be obtained. These values have been used to assess P limitation (Peters 1978), test phytoplankton nutrient competition theory based on cell size relationships (Smith and Kalff 1982), investigate causes of seasonal P_a variations in artifically fertilized lakes (Levine and Schindler 1980), and characterize seasonal variations in P_a and soluble-reactive phosphorus (SRP) concentrations in an oligotrophic lake (Tarapchak and Rubitschun 1981). However, Rigler's method has not been evaluated to determine if it yields a mathematically consistent upper bound on lake water P_a concentrations. In this report, we evaluate Rigler's method by identifying the parameters that control the shape of uptake curves

[1]This paper is partly based on a presentation at the P–Plankton Dynamics Symposium 27th Conference on Great Lakes Research, International Association for Great Lakes Research, May 1984, Brock University, St. Catharines, Ont., Canada.

[2]GLERL Contribution No. 506, Great Lakes Environmental Research Laboratory.

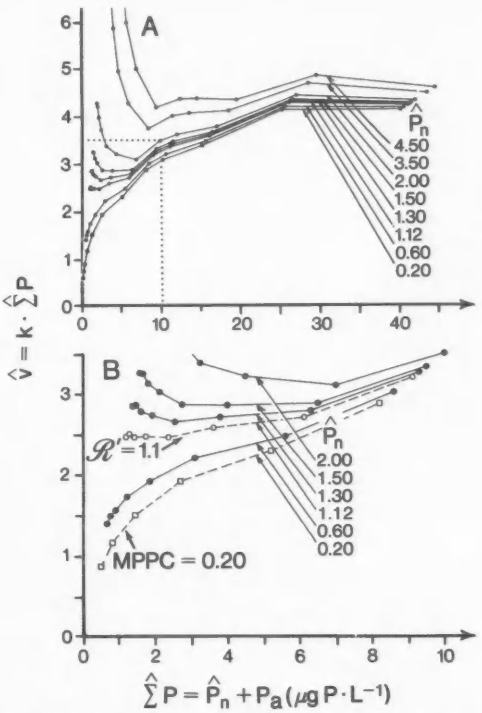

FIG. 1. Rigler plot for a P uptake experiment using offshore Lake Michigan water collected July 19, 1979. (A) Shapes of $\hat{v}$ versus $\hat{\Sigma}P$ curves as a function of $\hat{P}_n$ (curves truncated above $P_a = 40\ \mu g\ P \cdot L^{-1}$ for clarity). (B) Uptakes curves for the expanded part of Fig. 1A showing Rigler's MPPC estimate: $0.2\ \mu g\ P \cdot L^{-1}$ (open squares) and a gross upper bound estimate, $\mathscr{R}' = 1.1\ \mu g\ P \cdot L^{-1}$ (open circles).

in the bioassay, identify a new upper bound on P_a, and present a theoretical procedure to estimate limits on this bound in lake water experiments.

Rigler's Method

In Rigler's bioassay, subsamples of natural lake water are spiked simultaneously with radiolabeled orthophosphate (^{33}P) and different amounts of unlabeled orthophosphate (P_a). The rate constant, κ, for ^{33}P removal from solution in each subsample is measured and multiplied by the sum ($\hat{\Sigma}P$) of P_a and an assumed P_a concentration ($\hat{P}_n$) to yield uptake rate ($\hat{v}$) at each $\hat{\Sigma}P$. The circumflex indicates estimated or assumed rates and concentrations. This method assumes that the actual relationship of v to substrate concentration is hyperbolic and that v will be zero when ΣP is zero. To estimate MPPC, plots of $\hat{v}$ versus $\hat{\Sigma}P$ using successively lower assumed P_a concentrations are generated until the lower part of the uptake curve swings directly toward the plot's origin. Rigler proposed that the $\hat{P}_n$ value yielding this curve is MPPC. MPPC estimates in lakes range from values approximating chemically measured SRP to concentrations as low as $0.59 \times 10^{-4}\ \mu g\ P \cdot L^{-1}$ (Levine 1975; Levine and Schindler 1980).

Rigler's method is illustrated using results from a typical P uptake experiment using offshore Lake Michigan water (Fig. 1). This experiment was performed by adding P (as KH_2PO_4) to subsamples at final concentrations ranging from 0.08 to 150 $\mu g\ P \cdot L^{-1}$ and ^{33}P and estimating uptake rates (Tarapchak and Herche 1986). The SRP estimate obtained by the acid molybdate–stannous chloride method was $3.5\ \mu g\ P \cdot L^{-1}$ (Tarapchak and Rubitschun 1981). We chose $\hat{P}_n$ values greater and less than (following Rigler) this value to generate uptake curves, $\hat{v} = \kappa \cdot \hat{\Sigma}P$. The curves behave as predicted by Rigler, producing a series of concave upward (at relatively high $\hat{P}_n$) and concave downward (at relatively low $\hat{P}_n$) curves (Fig. 1A). To identify MPPC, uptake curves at relatively low $\hat{P}_n$ were carefully inspected to determine which curve approaches the plot's origin (Fig. 1B). A value of about $0.20\ \mu g\ P \cdot L^{-1}$ yields a curve directed toward the origin and represents the MPPC estimate in this experiment.

Evaluation of Rigler's Method

We examined Rigler's method by evaluating the parameters that control the behavior of uptake curves in $\hat{v}$ versus $\hat{\Sigma}P$ plots. Our analyses are based on the assumption that the relation of v to ΣP for a monospecific microbial population can be described by the Michaelis–Menten model

$$(1)\quad v = \frac{V_{max} \cdot S}{K_t + S}$$

where V_{max} is maximum uptake rate, K_t is the half-saturation constant (the substrate concentration at $V_{max}/2$), and S ($= \Sigma P$) is substrate concentration (Dugdale 1967; Button 1978). The symbol K_t is used instead of the Michaelis–Menten constant, K_m or K_s, to distinguish between nutrient uptake by cells or assemblages and substrate-dependent enzymatic reactions (Button 1985). For our analysis, we initially use a monospecific (one set of uptake constants) and a multispecific community (two or more sets of uptake constants), where uptake is described by a simple and a compound Michaelis–Menten equation, respectively (Tarapchak and Herche 1988). A "compound" model is necessary to describe uptake by communities composed of species with dissimilar K_t's because species uptake rates do not sum to a community rate described by the simple Michaelis–Menten model (Williams 1973; Tarapchak and Herche 1986) (see below). We examine the assumptions of Rigler's bioassay using simulated P uptake curves for hypothetical communities and demonstrate that the method fails to produce a consistently distinguishable estimate of MPPC.

Analytically, Rigler's method is based on empirical analyses of plots of $\hat{v}$ ($\kappa \cdot \hat{\Sigma}P$) versus $\hat{\Sigma}P$ for various assumed values of P_a ($\hat{P}_n$). The underlying assumption is that the experimentally measured quantity κ (a function of true ΣP) may be expressed as

$$(2)\quad \kappa = \frac{v}{\Sigma P} = \frac{V_{max}}{K_t + \Sigma P}$$

which gives

$$(3)\quad \hat{v} = \frac{V_{max} \cdot \hat{\Sigma}P}{K_t + \Sigma P}\ .$$

One may regard $\hat{P}_n$ as the sum of two components. The first is the true (and unknown) P_a concentration. The second, say Δ, is the difference between the estimate and the true value, so that $\hat{P} = P_a + \Delta$. To understand the behavior of equation (3), observe that, since $\hat{P}_n = P_a + \Delta$, $\Sigma P = P_a + P_a = \hat{P}_n + P_a$

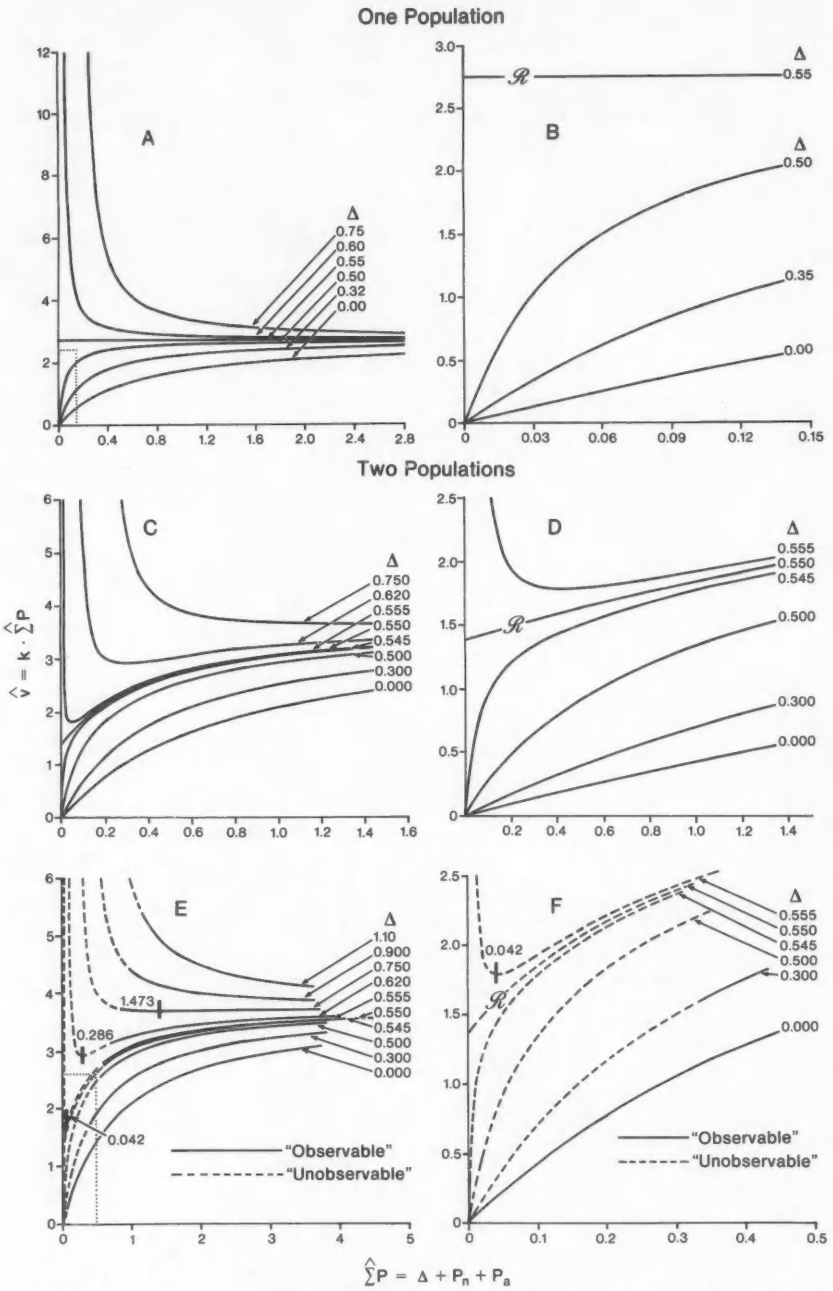

FIG. 2. Behavior of P uptake curves ($\hat{v}$ versus $\hat{\Sigma}P$) as a function of Δ for a (A and B) hypothetical monospecific and (C–F) bispecific community. All uptake curves for which $\Delta < K_s$ (monospecific community) or the lowest component species K_s (bispecific community) pass through the plot's origin. The $\mathcal{R}$ curve is produced by a P concentration equal to $K_{s(\min)}$ plus true P_n. Observable (solid lines) and unobservable (broken lines) regions of uptake curves are shown for the bispecific community over a $\hat{\Sigma}P$ domain of 0–5.0 μg P·L^{-1} (Fig. 2E) and 0–0.15 μg P·L^{-1} (Fig. 2F). Numbers at vertical marks indicate local minima (Fig. 2E and 2F). Monospecific parameters: V_{max} = 2.75 μg P·L^{-1}·h^{-1} and K_s = 0.55 μg P·L^{-1}. Bispecific parameters: V_{max} = 4.75 μg P·L^{-1}·h^{-1} and K_s = 0.95 μg P·L^{-1} plus those of the monospecific community. Populations of equal abundance. True P_n = 0.03 μg P·L^{-1} for both communities.

$- \Delta = \hat{\Sigma}P - \Delta$. Substituting the latter expression for ΣP in equation (3) yields

$$(4)\quad \hat{v} = \frac{V_{max}\cdot\hat{\Sigma}P}{K_t + (\hat{\Sigma}P - \Delta)} = \frac{V_{max}\cdot\hat{\Sigma}P}{(K_t - \Delta) + \hat{\Sigma}P}.$$

In equation (4), $K_t - \Delta$ functions as K_t in the standard Michaelis–Menten expression. The derivative, m, of $\hat{v}$ with respect to $\hat{\Sigma}P$ is

$$(5)\quad m = \frac{(K_t - \Delta)\,V_{max}}{[(K_t - \Delta) + \hat{\Sigma}P]^2}.$$

When Δ is larger than K_t, the quantities $K_t - \Delta$ and, consequently, m will be negative. This negative slope corresponds to an upward turn of the graph as $\hat{\Sigma}P$ becomes small. When Δ is smaller than K_t, the quantities $K_t - \Delta$ and m are positive and the plot assumes a "standard" downward concave shape. Hence, the curves generated in a Rigler plot are controlled by the relationship of Δ to K_t. This relationship allows us to examine the behavior of uptake curves using Δ instead of assumed P_n to determine if MPPC is a consistent estimator of an upper bound for P_n in lake water.

Rigler's criterion for estimating MPPC rests on the premise that some small assumed value of P_n yields a unique curve that extrapolates through the plot's origin. To test this premise, simulated uptake by two simple, arbitrarily constructed hypothetical communities is examined. The first is a monospecific community described by one set of uptake constants ($V_{max} = 2.75$ μg P·L^{-1}·h^{-1}, $K_t = 0.55$ μg P·L^{-1}); the second is a bispecific community described by two sets of uptake constants ($V_{max} = 2.75$ μg P·L^{-1}·h^{-1}, $K_t = 0.55$ μg P·L^{-1} and $V_{max} = 4.75$ μg P·L^{-1}·h^{-1}, $K_t = 0.95$ μg P·L^{-1}). We assumed a fixed (true) P_n concentration ($= 0.03$ μg P·L^{-1}) and generated curves for a standard Rigler plot (but with variation between curves determined by varying values of Δ). For the monospecific community, the family of curves gradually changes characteristically from concave upward to concave downward as Δ becomes smaller (Fig. 2A,2B), with the change in direction occurring when $\Delta = K_t$. Since $\hat{P}_n = P_n + \Delta$ is always chosen to be positive, Δ is always greater than $-P_n$.

Similar results are obtained for multispecific communities, except that more complex curves are generated. For example, curves that swing down near the y-axis can become locally concave up at low $\hat{\Sigma}P$. For the bispecific community (Fig. 2C,2D), all curves that assume a uniformly downward concave shape pass through the origin when Δ is less that the smallest K_t of a component species [$K_{t(min)}$]. However, we observed the same inconsistency in Rigler plots for both monospecific and multispecific communities — all curves for $\Delta < K_{t(min)}$ pass through the origin and all curves above do not. *Rigler's radiobioassay fails to produce an accurate estimate of an upper bound on P_n because all members of the family of curves with $-P_n < \Delta < K_{t(min)}$ (concave downward) pass through the plot's origin.* These curves all have the same general concave downward shape, becoming more shallow as Δ decreases. Because this analysis applies by analogy to multispecific lake water communities, Rigler's bioassay does not provide a consistently distinguishable upper bound on P_n in lake water.

Theoretical Upper Bound Identification

The analysis of uptake-curve behavior in Rigler plots provides a theoretical basis for identifying a consistently distinguishable upper bound on ambient P_n concentrations. This bound is the value of $\hat{P}_n$ for which $\Delta = K_{n(min)}$ (where the characteristic change in curve shape from concave upward to concave downward occurs). This value, termed Rigler ($\mathcal{R}$), is the sum of P_n and $K_{n(min)}$ (where $K_{n(min)}$ is positive by assumption). The $\mathcal{R}$ uptake curve produced by this value is the curve parameterized by the least value of $\hat{P}_n$ which does not go through the origin, i.e. intersects the positive y-axis. For the monospecific community, the $\mathcal{R}$ curve is easily identified because it is horizontal (Fig. 2A,2B). Here, $\mathcal{R}$ is equal to 0.550 [$K_{n(min)}$ plus 0.03 (P_n) = 0.580 μg P·L^{-1}. For the bispecific community, $\mathcal{R}$ corresponds to the curve with $\Delta = 0.550$ (Fig. 2C,2D) and is the sum of 0.550 [$K_{n(min)}$] and 0.03 (P_n) = 0.580 μg P·L^{-1}.

Detecting the Upper Bound in Lake Water

The above analysis has established that a consistently identifiable upper bound on P_n, $\mathcal{R}$, can be determined for samples in which $K_{n(min)}$ and P_n are known. We now address the obvious question — can $\mathcal{R}$ be determined in lake water samples composed of multispecific microbial assemblages and unknown P_n? As a basis for analysis, it should be recalled that uptake curves in Rigler plots have several features in common (see Fig. 2C,2D). First, the curves are essentially horizontal for large $\hat{\Sigma}P$. Second, for any Δ above $K_{n(min)}$, the curves have a vertical asymptote at $\hat{\Sigma}P = \Delta - K_{n(min)}$. Third, for any given curve where Δ is significantly $> K_{n(min)}$, the slope changes from (essentially) horizontal to (essentially) vertical as $\hat{\Sigma}P$ gets smaller. When the vertical portion of the curve can be discerned, Δ will always be above $K_{n(min)}$. Fourth, for small $\Delta > K_{n(min)}$, the curves slope down as $\hat{\Sigma}P$ becomes small before they sweep up and approach vertical.

One desires to identify the curve corresponding to $\mathcal{R}$ in lake water experiments. To do this, it is necessary to see the left end of the family of curves change shape from concave up (approaching a left vertical asymptote) to concave down (going through the origin) as $\hat{P}_n$ gets smaller. However, it is not possible to have data corresponding to the left end of the curve because $\hat{\Sigma}P$ must be greater than or equal to $P_n + \Delta = \hat{P}_n$. Consequently, there will be an "observable" and an "unobservable" region (domain) for a P uptake curve, given a particular Δ. Each curve is observable on the domain $\hat{\Sigma}P \geq P_n + \Delta$, i.e. when $P_a \geq 0$. For example, in the bispecific community (Fig. 2E), $\hat{v}$ is observable for all points with $\hat{\Sigma}P$ greater than 0.03 (P_n) plus 0.62 (Δ) (i.e. $\hat{\Sigma}P \geq 0.65$) (curve No. 4 from top). The observable portion of each curve is shown as a solid line. Conversely, the portion of each curve with $\hat{\Sigma}P < P_n + \Delta = 0.65$ would necessarily correspond to data with $P_a = \hat{\Sigma}P - (P_n + \Delta) < 0$, which is not realisable (i.e. data cannot be obtained with negative P_a). Hence, this portion of each curve, shown as a broken line, is unobservable. Note that some portion of all the uptake curves for the bispecific community will not be observable (Fig. 2E).

These analyses show that estimating true $\mathcal{R}$ in natural lake water samples will be complicated by the fact that the features identifying the $\mathcal{R}$ curve are obscured by being in the unobservable region. This can be illustrated as follows (Fig. 2E,2F). Note that as Δ approaches $K_{n(min)}$ from above, the local minima where the slopes of the curves change sign have abscissae that approach zero. These points with zero slope are identified on the curves with vertical marks. When Δ equals $K_{n(min)}$, the abscissa of the minimum reaches the axis and the curves become uniformly concave downward. As the minimum point

approaches the axis, its abscissa becomes less than $P_n + \Delta$ and is then unobservable. Observe that for $\Delta = 0.75$, the minimum (at 1.473) is above $\hat{\Sigma}P = 0.75$ plus 0.03 μg P·L^{-1} and is observable. For $\Delta = 0.62$, the minimum is below $\hat{\Sigma}P = 0.62$ plus 0.03 μg P·L^{-1} and is unobservable. Thus, one cannot observe the behavior of curves closer than $\hat{P}_n$ to the *y*-axis. Hence, the curve parameterized by $\hat{P}_n$ equal to $\mathcal{R}$ cannot be distinguished in lake water samples.

Lake Water Experiment: Estimates of an Upper Bound

We now develop a relationship used for setting limits on $\mathcal{R}$ in natural samples. The approach requires an experimentally estimable bound, $\mathcal{R}'$, on $\mathcal{R}$. Then we establish a relationship between true $\mathcal{R}$, the bound $\mathcal{R}'$ and P_n (see Theoretical Limits on $\mathcal{R}$ in Lake water Experiments (next section)). Here we identify the bound $\mathcal{R}'$ and its method of estimation. In theory, the only visually distinguished curve in a lake water experiment is the last curve (as Δ gets smaller) for which the local minimum is observable. Curves below this cannot be qualitatively distinguished because as $\hat{P}_n$ becomes smaller, the minima of the curves go into the unobservable region. The value of $\hat{P}_n$ for this curve is termed $\mathcal{R}'$. For a multispecific community, we expect that the $\mathcal{R}'$ curve would "tail up" to the left of $\hat{\Sigma}P = \mathcal{R}'$ (see Fig. 2E,2F). By comparison, note that among the curves with $\hat{P}_n < \mathcal{R}$, the observable portions of the curves with bigger Δ's are indistinguishable from those below them. The value of $\hat{P}_n$ parameterizing the $\mathcal{R}'$ curve is an upper bound for both true $\mathcal{R}$ and P_n.

The procedure for obtaining $\mathcal{R}'$ in a natural sample is illustrated in Fig. 1. This bound for ambient true P_n is the value of $\hat{P}_n$ producing a curve in a Rigler plot that is horizontal near the *y*-axis, i.e. a curve which is about to curl down from the horizontal for the smallest values of $\hat{P}_n$. To obtain the estimate, we first generate a set of curves using trial values of $\hat{P}_n$ and select curves that appear to be near horizontal at their left end (Fig. 1B). We then use a richer set of values to empirically identify the value of $\hat{P}_n$ that yields a line whose slope there does not differ from zero. For this experiment, $\mathcal{R}'$ is approximately 1.1 μg P·L^{-1}.

Theoretical Limits on $\mathcal{R}$ in Lake Water Experiments

A theoretical rationale for setting upper and lower limits on $\mathcal{R}$ in lake water samples can be developed from the foregoing analyses. The approach is based on finding the relationship between $\mathcal{R}$ and $\mathcal{R}'$ for various hypothetical communities and values of P_n. The approach assumes that the abundance-weighted species K_t distribution in lake water is known. It should be noted that $\mathcal{R} = P_n + K_{t(\min)}$. Consequently, it is possible to get only limits on $\mathcal{R}$ due to the fact that when $\Delta = K_{t(\min)}$, $\hat{\Sigma}P = P_n + K_{t(\min)}$ is the smallest observable datum, while minima for nearby curves would have unobservable abscissae less than $P_n + K_{t(\min)}$. To find $\mathcal{R}'$ for a given multispecific assemblage and assumed true P_n, one needs to find $\hat{P}_n$ such that the minimum of the curve parameterized by $\hat{P}_n$ falls on the limit of observability, namely $\hat{P}_n$. This may be accomplished by numerically searching for the value of $\hat{P}_n$ (given P_n) which satisfies the relationship.

One way of relating $\mathcal{R}'$ to $\mathcal{R}$ is to examine the ratio of the difference, $D = \mathcal{R}' - \mathcal{R}$ to $\mathcal{R}$ for each value of assumed P_n. Our analyses were performed by assuming that true P_n is less than some nominal P_n bound. The nominal bound was arbitrarily set at $P_n < 5$ μg P·L^{-1}. For a given hypothetical community, the ratio $D/\mathcal{R}$ has a minimum lower limit (L) and a maximum upper limit (U) for this domain of P_n, giving

$$(6) \quad \frac{D}{\mathcal{R}} \geq L$$

and

$$(7) \quad \frac{D}{\mathcal{R}} \leq U.$$

From equation (6), $D \geq L \cdot \mathcal{R}$, and so, using $D = \mathcal{R}' - \mathcal{R}$, one sees that

$$\mathcal{R}' = \mathcal{R} + D \geq \mathcal{R} + L \cdot \mathcal{R} = \mathcal{R}(1 + L)$$

or

$$\mathcal{R} \leq \frac{\mathcal{R}'}{1+L},$$

an upper bound on $\mathcal{R}$ in terms of $\mathcal{R}'$. Similarily, from equation (7):

$$D \leq U \cdot \mathcal{R} \text{ implies} \mathcal{R} \geq \frac{\mathcal{R}'}{1+U},$$

a lower bound on $\mathcal{R}$ in terms of $\mathcal{R}'$. Together:

$$(8) \quad \frac{\mathcal{R}'}{1+U} \leq \mathcal{R} \leq \frac{\mathcal{R}'}{1+L}.$$

This gives upper and lower limits for $\mathcal{R}$ in lake water samples in terms of $\mathcal{R}'$ under the assumption that the lake water assemblage is identical to the hypothetical community. The value for $\mathcal{R}'$ in a lake water experiment is obtained and applied in equation (8).

Calculating Limits on $\mathcal{R}$ in Lake Water Experiments

To illustrate this procedure for estimating true $\mathcal{R}$ in a natural sample, we constructed multispecific microbial communities and simulated P uptake in Rigler plots. Knowledge of the range of species K_t's and the abundance-weighted K_t distributions in natural P-limited assemblages is meager (Rhee and Gotham 1980; Tilman 1981; Tarapchak and Herche 1986). Michaelis–Menten uptake constants are known for only a few naturally occurring microorganisms (Stross and Pemrick 1974; Smith and Kalff 1982). To mimic the structure of natural assemblages, we constructed hypothetical communities consisting of 30 species and assigned species K_t values guided by our knowledge of assemblage K_t estimates for Lake Michigan (Tarapchak and Herche 1986) rather than literature values from laboratory monoculture studies (Nalewajko and Lean 1980). Thirty species were used, since autoradiographic studies on ^{33}P uptake by natural Lake Michigan microbial assemblages frequently revealed that 30 species took up at least 98% of the ^{33}P. Half-saturation constants ranging from extremes of 0.05 to 50 μg P·L^{-1} were assigned to species. Species abundances were set equal or permitted to range up to 1000-fold in "skewed" species K_t-abundance arrangements (Tarapchak and Herche 1986).

TABLE 1. Communities consist of 30 species divided equally among two groups (Communities I–IV) or in a single group (Community V). Species K_t values are uniformly spaced within each order of magnitude. Relative species abundances are distributed 80% (first group) and 20% (second group) for Communities I–IV, where within-group species abundances are set equal (Equal) or are uniformly spaced within two orders of magnitude. P_n ambient orthophosphate concentration, $\mathcal{R}$ a true P_n upper bound, $\mathcal{R}'$ a visually estimable upper bound on $\mathcal{R}$, and calculated limits L (lower) and U (upper) on $D/\mathcal{R}$. Units are μg P·L^{-1} except for the ratio.

Community	Species K_t range	Relative species abundance	P_n = 0.21 μg P·L^{-1}			0 < P_n < 5	
			$\mathcal{R}$	$\mathcal{R}'$	$(\mathcal{R}'-\mathcal{R})/\mathcal{R}$	L	U
IA	(0.05–0.5) (5–50)	Equal (1)	0.260	0.708	1.721	1.451	3.442
IB	(0.05–0.5) (5–50)	(1000–10) (100–1)	0.260	0.469	0.802	0.379	2.577
IC	(0.05–0.5) (5–50)	(10–1000) (1–100)	0.260	3.820	13.70	1.341	59.63
IIA	(0.05–5) (5–50)	Equal (1)	0.260	0.476	0.829	1.211	13.07
IIB	(0.05–5) (5–50)	(1000–10) (100–1)	0.260	0.469	0.802	0.379	2.577
IIC	(0.05–5) (5–50)	(10–1000) (1–100)	0.260	3.820	13.70	1.341	59.63
III	(0.5–5) (5–50)	Equal (1)	0.711	2.771	2.900	0.995	3.367
IV	(0.05–0.5) (0.5–5) (5–50)	(1000–100) (100–10) (10–1)	0.260	0.487	0.872	0.277	1.710
V	(0.1–100)	(1000–1)	0.310	7.687	23.79	4.747	24.00

Simulations are based on the assumption that P uptake for each species in the community can be adequately described by the simple Michaelis–Menten model (Button 1978), where uptake is described using pairs of uptake constants (K_t, V_{max}) which differ among species. Using subscript j to denote parameters for any one (jth species), equation (4) was generalized to describe uptake rate, v, for the entire n-species community as the sum of individual species uptake rates:

$$v = \sum_{j=1}^{n} \frac{a_j \cdot V_{max_j} \cdot \hat{\Sigma}P}{K_{t_j} + (\hat{\Sigma}P - \Delta)}$$

$$= \sum_{j=1}^{n} \frac{a_j \cdot V_{max_j} \cdot \hat{\Sigma}P}{(K_{t_j} - \Delta) + \hat{\Sigma}P}$$

where each species is weighted by its relative abundance (fraction of biomass), a_j, in the community. Ambient true P_n was set at 0.21 μg P·L^{-1} to mimic the median of the Rigler estimates for the spring–early summer period in Lake Michigan.

Simulated P uptake for a variety of hypothetical multispecific communities shows the expected complex behavior of uptake curves in Rigler plots and that $\mathcal{R}'$ significantly exceeds $\mathcal{R}$ as predicted (Table 1). A Rigler plot for Community IA is shown to illustrate results (Fig. 3). For this community, $\mathcal{R}'$ by inspection would be approximately 0.21 + 0.5 = 0.71 μg P·L^{-1} (shown as the $\mathcal{R}'$ curve in Fig. 3A). However, $\mathcal{R}$ is 0.21 + 0.05 = 0.26 μg P·L^{-1} (Fig. 3B). Note that $\hat{P}_n$ values near $\mathcal{R}$ produce curves that swing up in the unobservable region (Fig. 3B). This behavior would not be discerned in a lake water experiment, as argued above, based on P uptake by the hypothetical bispecific community (Fig. 2). These results argue generally that the true upper bound for ambient P_n in lake water will not be closely approximated by Rigler estimates (Fig. 1). The only identifiable upper bound in a lake water sample is $\mathcal{R}'$ which can grossly overestimate $\mathcal{R}$.

The hypothetical community (Community IA) in Fig. 3 is used to illustrate how limits on $\mathcal{R}$ are obtained for a lake water sample. The relationships of $\mathcal{R}$, $\mathcal{R}'$, $D := \mathcal{R}' - \mathcal{R}$, and $D/\mathcal{R}$ for P_n in the domain of 0–5 μg P·L^{-1} are shown in Fig. 4. At P_n = 0.21 μg P·L^{-1}, $\mathcal{R}$ = 0.26, $\mathcal{R}'$ = 0.71, D = 0.45, and $D/\mathcal{R}$ = 1.73. $D/\mathcal{R}$ has a maximum value of 3.442 and a minimum value of 1.451. Assuming that the hypothetical community represents the lake water assemblage:

$$\frac{\mathcal{R}'}{1+U} = \frac{\mathcal{R}'}{1+3.442} = \frac{\mathcal{R}'}{4.442}$$

and

$$\frac{\mathcal{R}'}{1+L} = \frac{\mathcal{R}'}{1+1.451} = \frac{\mathcal{R}'}{2.451}$$

or

$$\frac{\mathcal{R}'}{4.442} \leq \mathcal{R} \leq \frac{\mathcal{R}}{2.451}.$$

Using the observed $\mathcal{R}'$ value of 1.1 μg P·L^{-1} from the lake water experiment (Fig. 1), the value of $\mathcal{R} = P_n + K_{t(min)}$ is bounded as

$$\frac{1.1}{4.442} \leq \mathcal{R} \leq \frac{1.1}{2.451}$$

or

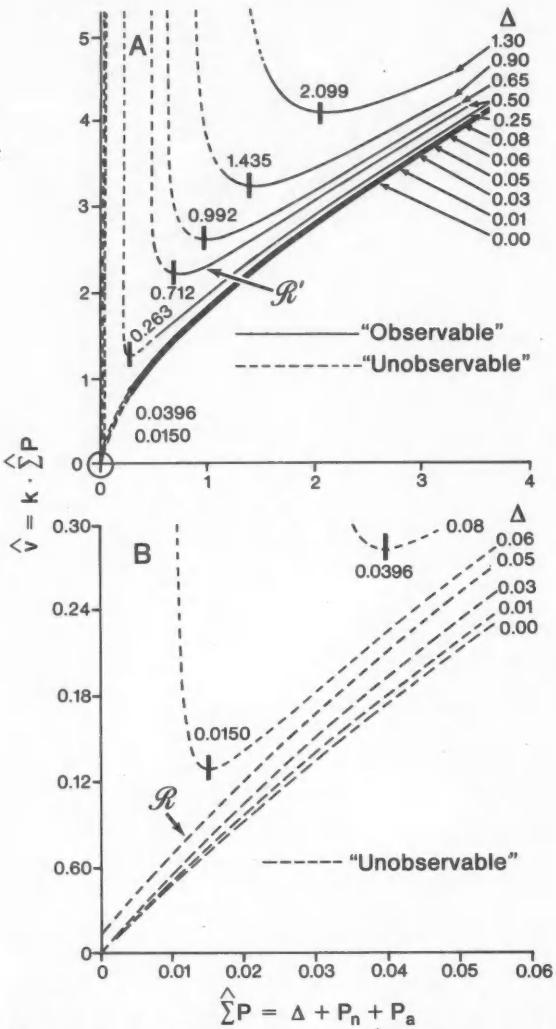

FIG. 3. Behavior of P uptake curves ($\hat{v}$ versus $\hat{\Sigma}P$) as a function of Δ for a hypothetical community consisting of 30 species showing the observable (solid lines) and unobservable (broken lines) uptake regions (Community IA, see Table 1). Numbers at vertical marks indicate local minima. The feature of note in Fig. 3A is the $\mathcal{R}'$ curve; the feature on Fig. 3B (expanded part of Fig. 3A indicated by circle) is the $\mathcal{R}$ curve. True assumed $P_n = 0.21$ μg P·L^{-1}. Community consists of two microbial groups with the K_s's of species ranging from 0.05 to 0.5 μg P·L^{-1} (first group) and 5–50 μg P·L^{-1} (second group).

$$0.2476 \leq \mathcal{R} \leq 0.448.$$

These limits are substantially below the estimate of $\mathcal{R}'$ (1.1. μg P·L^{-1}) in the lake water experiment.

These limits on $\mathcal{R}$ are nonconservative, since they rely completely on the community structure described for Community IA. A more conservative approach is to assume that any one of the hypothetical communities in Table 1 might describe the structure of the natural assemblage. Hence, a conservative estimate of L and U for the lake water sample is the minimum of the L's and the maximum of the U's for all candidate communities. From inspection, the values of 0.277 and 59.6 are

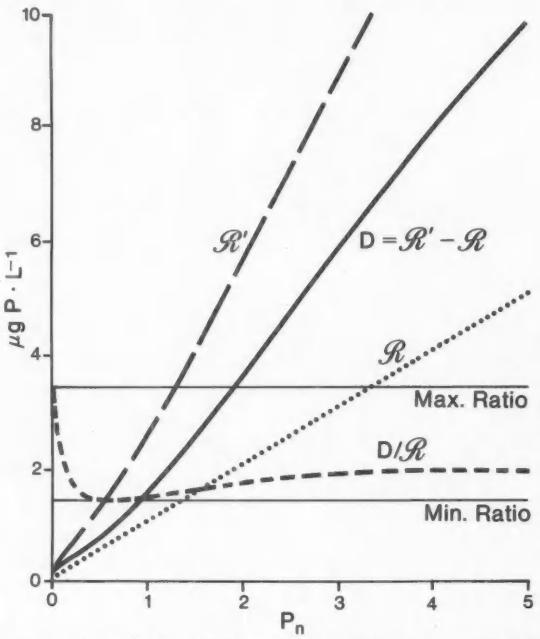

FIG. 4. Values of $\mathcal{R}$, $\mathcal{R}'$, $D = \mathcal{R}' - \mathcal{R}$ (μg P·L^{-1}) and $D/\mathcal{R}$ (dimensionless) over the P_n domain of 0–5 μg P·L^{-1} for the hypothetical community in Fig. 3 used to obtain U and L. Horizontal lines indicate the minimum (1.451) and maximum (3.442 μg P·L^{-1}) of the $D/\mathcal{R}$ ratio over the substrate domain.

the minimum of the lower ratio limit and the maximum of the upper ratio limit, respectively (Table 1). Substituting these values into equation (8) (and using $\mathcal{R}' = 1.1$ μg P·L^{-1}) gives

$$1.1/(1 + 59.63) \leq \mathcal{R} \leq 1.1/(1 + 0.277)$$

or

$$0.018 \leq \mathcal{R} \leq 0.861.$$

These estimates are more robust than those obtained from using an individual hypothetical community (Table 1). This upper limit on $\mathcal{R}$ is slightly below the experimental $\mathcal{R}'$ value.

Discussion and Conclusions

Methods of measuring ambient true P_n concentrations in aquatic systems are in an embryonic state of development (Rigler 1973; Tarapchak and Nalewajko 1986). Three important insights into setting bounds on P_n concentrations in lake water have arisen from our analysis. First, Rigler's MPPC estimates do not yield valid upper bound P_n values. Any uptake curve in a Rigler plot, where the error in estimating P_n is less than $K_{s(\min)}$, yields a candidate for MPPC, since the curve will intersect the plot's origin. Rigler's MPPC estimates must now be interpreted as $\hat{P}_n$ values located somewhere between a true upper bound P_n value and zero. Presently, there is no way of determining how closely MPPC approximates true P_n, as generally accepted independent methods for P_n estimation are unavailable.

Second, a new upper bound on true P_n, $\mathcal{R}$, has been identified. This bound, defined as the sum of P_n and $K_{s(\min)}$, can be calculated for any multispecific assemblage when P_n and the

lowest species K_t are specified. In theory this value satisfies an important requirement in P_n estimation. $\mathcal{R}$ is a mathematically determined value based on parameters controlling P uptake curve behavior in Rigler plots. Unfortunately, the $\mathcal{R}$ curve cannot be distinguished in a lake water experiment where species have dissimilar K_t's because of the complex behavior of uptake curves in the unobservable substrate region. The next step in these analyses must be to determine how strongly the assumptions on community structure (e.g. species K_t ranges, V_{max} to K_t ratios, and abundance-weighted K_t's) affect the $\mathcal{R}/\mathcal{R}'$ relationship and the limits on $\mathcal{R}$. We have not attempted these analyses here because of the present uncertainty in the actual structure of natural communities (Smith and Kalff 1982; Tarapchak and Herche 1986).

Third, the theoretical framework for setting limits on $\mathcal{R}$ by relating the P uptake kinetics by natural and hypothetical communities provides new insights into upper bound P_n estimation. The procedure hinges on the assumption that the ratio of $\mathcal{R}$ to $\mathcal{R}'$ for the hypothetical community is the same as for the lake water assemblage. $\mathcal{R}$ is invariant for communities with the same P_n and $K_{t(\min)}$. However, $\mathcal{R}'$ estimates for a given community are highly variable depending on the abundance-weight species K_t distribution. For example, rearranging the species abundance distribution so that species with the lowest K_t's are assigned the lowest (Community IC) rather than the highest (Community IB) abundance value changes the $\mathcal{R}'$ value and L and U by an order of magnitude. Although one may obtain reasonable estimates of $K_{t(\min)}$ by direct experimental measurement (Smith and Kalff 1982) or by inference from natural microbial assemblage K_t estimates (Tarapchak and Herche 1986), the range of species K_t values and the abundance-weighted species K_t distributions in natural systems are poorly known. Consequently, although the procedure is an attractive means of estimating limits on $\mathcal{R}$, the method must be used cautiously until species-specific uptake rates for entire microbial assemblages in particular systems are better known.

In summary, P uptake experiments using microbial assemblages from P-limited systems do not yield a consistently distinguishable upper bound on ambient true P_n concentrations. The only identifiable bound, $\mathcal{R}'$, is empirical and probably grossly overestimates true $\mathcal{R}$. The theoretical procedure for estimating limits on true $\mathcal{R}$ is strongly dependent on hypothetical species K_t-abundance distributions. Presently, the method can be used to set limits on $\mathcal{R}$ in systems where there is some knowledge of possible species K_t-abundance distributions. Bounded $\mathcal{R}$ values can be used to test predictions on seasonal variations in P_n concentrations emanating from complex mathematical ecosystem models. However, our estimates on $\mathcal{R}$ bounds are insufficiently conservative to be used in aquatic chemical equilibrium studies and process research in phosphorus–plankton dynamics.

Acknowledgements

This work was motivated by stimulating discussions with Frank Rigler. H. Vanderploeg, P. Landrum, B. Eadie, G. Stehly, J. Cotner, and W. Gardner provided helpful comments on the manuscript.

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PERSPECTIVES

Tests of Competition between Native and Introduced Salmonids in Streams: What Have We Learned?

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Fausch, K. D. 1988. Tests of competition between native and introduced salmonids in streams: what have we learned? Can. J. Fish. Aquat. Sci. 45: 2238–2246.

Salmonids have been introduced worldwide to regions where they were not indigenous, usually without considering the effects on native species. This has led to many combinations of species in unnatural sympatry, some of which are suspected to interact strongly, but we currently have inadequate knowledge to allow us to predict the outcome of most such introductions. For exampie, despite current controversy about the potential effects of Pacific salmonids now stocked in Atlantic waters, we have only empirical evidence with which to judge whether they will be detrimental to Atlantic salmon (*Salmo salar*). Moreover, most experimental tests of interspecific competition between other pairs of stream salmonids have not been rigorous. A review of literature about the suspected competition between brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) in streams reveals that despite empirical evidence that water temperature and velocity play a critical interacting role in determining the outcome, most experiments have failed to elucidate the important mechanisms involved. Carefully designed experiments that compare the strength of interspecific versus intraspecific competition for different size classes will allow us to improve predictions about the effects of introduced salmonids on native species in streams.

Partout dans le monde, on a introduit des salmonidés dans des régions où ils n'étaient pas indigènes, habituellement sans considération des effets qu'un tel changement pourrait avoir sur les espèces d'origine. Par suite de ces introductions, il s'est formé un grand nombre de combinaisons sympatriques artificielles entre diverses espèces, combinaisons dont certaines sont présumées mettre en jeu des interactions fortes; toutefois, dans la plupart des cas, l'état actuel de nos connaissances ne nous permet pas de prévoir les effets qui peuvent s'ensuivre. Par exemple, en dépit de la controverse au sujet des effets que peuvent avoir les opérations d'ensemencement actuelles par lesquelles on introduit des salmonidés du Pacifique dans l'Atlantique, nous ne pouvons déterminer qu'empiriquement si le saumon de l'Atlantique (*Salmo salar*) s'en ressentira. En outre, la plupart des expériences de compétition interspécifique mettant en jeu d'autres couples de salmonidés dulcicoles n'ont pas été réalisées de façon rigoureuse. Si l'on regarde ce qui se dit dans la littérature au sujet de la compétition présumée entre l'omble de fontaine (*Salvelinus fontinalis*) et la truite arc-en-ciel (*Salmo gairdneri*) dans les cours d'eau, on constate que même si l'on sait de façon empirique que la température et la vitesse de l'eau sont des facteurs interactifs dont l'influence est déterminante, on voit que la plupart des expériences n'ont pas permis de comprendre les grands mécanismes mis en jeu. Des expériences soigneusement élaborées dans lesquelles on pourra comparer l'intensité de la compétition interspécifique et intraspécifique de différentes classes de taille nous permettront de mieux prévoir les effets que peut avoir l'introduction de salmonidés sur les espèces d'origine dans les cours d'eau.

Received March 9, 1988
Accepted August 10, 1988
(J9632)

Reçu le 9 mars 1988
Accepté le 10 août 1988

Many species of salmonids have been introduced throughout the world to regions where they were not indigenous, generally with little regard to the effects on native species. Investigations of the recorded introductions for a given region often reveal that most common species of salmonids have been introduced there during the last 125 yr (e.g. McDowall 1984; Wiltzius 1985), although less than half are likely to have been successful in establishing reproducing populations. For instance, between 1873 and 1972, 13 species of salmonids were introduced into waters of the state of Michigan (Latta 1974), although only 6 were considered successful and only 3 of these would be widely distributed in the absence of stocking. Even Kerguelen, a small barren archipelago in the remote southern Indian Ocean owned by France, has received brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), rainbow trout (*Salmo gairdneri*), coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*), and Atlantic salmon (*Salmo salar*), all since 1955 (Sagar 1986).

These introductions lead to a large number of possible sympatric combinations in streams. Using North America as an example, if one considers only relatively common salmonid species that typically reside in streams for more than a few weeks of life, one can generate a list of 13 species (Robins et al. 1980) (Table 1) which taken 2 at a time yields 78 combi-

TABLE 1. Thirteen relatively common salmonid species of North America that typically reside in streams for more than a few weeks of their life cycle (based on Salmonidae listed in Robins et al. 1980). Calculation of the number of possible sympatric pairs of these 13 species is shown below.

Oncorhynchus kisutch	*Salmo salar*
Oncorhynchus tshawytscha	*Salmo trutta*
Prosopium williamsoni	*Salvelinus alpinus*
Salmo aguabonita	*Salvelinus confluentus*
Salmo clarki	*Salvelinus fontinalis*
Salmo gairdneri	*Salvelinus malma*
	Thymallus arcticus

$$13 \text{ species taken 2 at a time} = \frac{13!}{2!\cdot 11!} = 78 \text{ combinations}$$

nations. A few species generally occupy different habitats due to physiological constraints (e.g. *Thymallus arcticus* and *S. trutta*), which would tend to decrease the number of combinations, but others have many ecologically distinct forms (e.g. *Salmo clarki*, Behnke 1979), which would tend to increase it. In any event, a large number of sympatric combinations of salmonids are likely to occur in lotic systems worldwide.

Because most of these pairs of species did not evolve in sympatry, there has been no opportunity for natural selection to produce differences in resource use that would ameliorate competition. It is therefore likely that some pairs will compete strongly for limited resources. For instance, several subspecies of cutthroat trout, including the westslope (*Salmo clarki lewisi*), greenback (*S. c. stomias*), and Lahontan (*S. c. henshawi*) forms, do not persist long in western North American streams after brook or brown trout are introduced and are suspected to interact strongly with these non-native species (MacPhee 1966; Behnke 1979; Moyle and Vondracek 1985; Gerstung 1988; but see Griffith 1972, 1988).

But have we gained enough knowledge to allow us to predict which combinations of unnatural sympatry will be ecological disasters and which will be relatively benign (Taylor et al. 1984; Pimm 1987)? I believe that, for stream salmonids, the answer is generally no. One reason is that studies designed to determine the effect of an introduced salmonid have often generated equivocal results due to inadequate experimental design or execution. Hence, I first show by way of an example from the Atlantic coast of North America why we need better information on salmonid interactions. I then critically evaluate attempts to measure interspecific competition among stream salmonids in field and laboratory studies, based primarily on a case study of research for one pair of species, brook and rainbow trout. I conclude by summarizing the important questions generated by this research and then offer advice about factors to consider when designing experiments to test competition among stream salmonids.

Although predation by introduced salmonids may also have important effects on other salmonid species (Alexander 1977, 1979), I choose to emphasize competition because it has been relatively well studied, by comparison. This perspective differs from Hearn's (1987) broad overview of interspecific competition and habitat segregation among stream salmonids in that I critically review research about specific sets of interacting species. I hope my observations will encourage others to develop reliable knowledge on which to base future predictions about the effects of introduced salmonids.

Will Pacific Salmonids Be Detrimental to Atlantic Salmon?

A good example that illustrates why we need better information on interspecific competition is the continued introduction of salmonids native to the Pacific coast of North America into Atlantic waters. Fish ecologists and fisheries managers on both sides of the Atlantic Ocean have been wondering what effects these exotic species, especially coho salmon and rainbow trout (or steelhead, the anadromous form) which rear the longest in streams, will have on Atlantic salmon and other native salmonids (Berg 1977; Gruenfeld 1977; Solomon 1979; Chadwick and Bruce 1981; Gibson 1981; Randall 1984; Shaw 1985; Porter and Goldthwaite 1987; Dumont et al. 1988).

The potential for interspecific competition among these salmonids appears high. Atlantic salmon juveniles are more abundant in riffles during summer, especially in the presence of pool-dwelling competitors such as brook and brown trout (Gibson 1966, 1978; Kennedy and Strange 1986). Thus, one might expect interspecific competition to be strongest between Atlantic salmon and steelhead because juvenile steelhead use primarily riffles during summer (Hartman 1965) and both species overwinter in streambed interstices (Bustard and Narver 1975; Rimmer et al. 1983, 1984). But the evidence suggests that Atlantic salmon may also shift to use pools during winter, as they grow larger, or when competitors are absent (Gibson 1966, 1978, 1981; Kennedy and Strange 1986). Competition with coho salmon, which primarily inhabit pools (Hartman 1965; Glova 1986), may therefore become important for older juvenile Atlantic salmon, and for all age classes of juveniles during the winter.

This potential competition is an important problem, given the costly efforts by fishery managers to restore Atlantic salmon in northeastern North America (Fay 1978; Moffitt et al. 1982; Stolte 1986) while simultaneously introducing coho salmon in the Great Lakes (Tody and Tanner 1966; Withler 1982) and various New England states (Stolte 1974; Martin and Dadswell 1983). Others are experimenting with ocean pen rearing of coho along the Atlantic coast (Symons and Martin 1978; Porter and Goldthwaite 1987) and off France (Solomon 1979; Gras et al. 1983). As a result of these introductions, Quebec fish biologists (Dumont et al. 1988) have reported sporadic captures of adult coho and chinook salmon since 1970 throughout the St. Lawrence River system and have observed Pacific salmon spawning near Montreal. Coho salmon have reproduced most years since 1978 in one Nova Scotia river (Porter and Goldthwaite 1987) although that population now appears to be waning (S. E. Barbour, Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S. B3J 2S7, pers. comm.).

Steelhead have been reproducing in the Great Lakes since the early 1900's and rainbow trout are widely introduced in Atlantic coast provinces and states (Porter and Goldthwaite 1987; Dumont et al. 1988). Rainbow trout of 25–40 cm are captured relatively frequently throughout the St. Lawrence River (Whoriskey et al. 1981; Dumont et al. 1988) and breeding populations are reportedly established as far away as western Newfoundland (Chadwick and Bruce 1981).

Given the potential for damage to native species, should introductions of Pacific salmonids be curtailed or banned in Atlantic waters, except perhaps for sterile fish used in cage culture? The problem is that we have little experimental evidence with which to evaluate the effects of these introductions on Atlantic salmon (see Gibson 1981).

Lacking experimental evidence, we are forced to evaluate the probable effects of introduced salmonids based on empirical (i.e. observational) evidence alone. R. J. Behnke (Department of Fishery and Wildlife Biology, Colorado State University, Ft. Collins, CO 80523, pers. comm.) suggests that anadromous Pacific salmonids pose little threat because attempts to establish reproducing populations of pink salmon (*Oncorhynchus gorbuscha*) in Maine (Porter and Goldthwaite 1987), Newfoundland (Lear 1975), and the White Sea of the Arctic Ocean (Persov et al. 1983; Agapov 1986) have failed, even after introducing large numbers of fish for many years. Possible reasons given for the failures include disruption of homing due to different currents, magnetic fields, or sun orientation in the Atlantic Ocean (Lear 1975; R. J. Behnke, pers. comm.; see also Stewart 1977), predation on fry in rivers and the ocean (Lear 1975), and unfavorable river temperatures during spawning and incubation (Persov et al. 1983; Agapov 1986) or smolting (Lear 1975). Behnke also points out that besides the Laurentian Great Lakes, New Zealand is one of the only places anadromous Pacific salmonids, in this case chinook salmon, have established reproducing populations outside their native range, probably due to stocking millions of fish from different sources during 1875–1912 (Stokell 1955; McDowall 1984).

I tend to take a more conservative view of the potential for damage to Atlantic salmon stocks. Despite the failure of introduced Pacific salmonids elsewhere, four species have successfully established adfluvial populations in the Great Lakes. Pink salmon accidentally released in Lake Superior in 1956 produced populations that spread to all the Great Lakes by 1979 (Emery 1981; Kwain and Lawrie 1981). Steelhead, coho salmon, and chinook salmon are maintained primarily by stocking, but straying spawners produce substantial wild populations in favorable environments (Peck 1970; Patriarche 1980; Carl 1982). Thus, continued accidental or intentional introductions of Pacific salmonids may yet be successful in Atlantic waters, especially where native fish populations or their habitat are perturbed. I therefore believe that the question of competition between coho salmon or steelhead and Atlantic salmon deserves careful research (cf. Porter and Goldthwaite 1987; Dumont et al. 1988).

Experiments that Test Interspecific Competition

Despite the long history and large number of non-native salmonid introductions, very few investigations provide strong evidence for or against interspecific competition with native species in natural streams. Most such studies of stream salmonids can be criticized on at least one of three points. First, many field studies are based strictly on observations of species in sympatry and allopatry at different locations and hence lack experimental manipulation, such as Griffith's (1972) research on brook and westslope cutthroat trout in Idaho and Cunjak and Green's (1983) work on brook and rainbow trout in Newfoundland. Without manipulation, one cannot determine whether a niche shift or ecological release by a species in the absence of its competitor is not simply an artifact of differences between streams or sites (Connell 1975; Sale 1979), unless other supporting evidence is assembled for a large number of sites (see Diamond 1986). I should point out that both Griffith (1972) and Cunjak and Green (1983) set out to study habitat use rather than to test interspecific competition, yet both also used their results to infer whether competition is important or not.

TABLE 2. Experimental design to test interspecific and intraspecific competition between two species (after Underwood 1986, table 11.2). Numbers refer to relative densities of each species. The effect of interspecific competition is tested by comparing treatments where a species is allopatric versus those where an equal density of its competitor is added (treatment 2 vs. 3 for species A and treatment 4 vs. 3 for B). By comparison, the effect of intraspecific competition is tested by comparing low-density allopatric treatments with those where an equal density of conspecifics is added (treatment 2 vs. 1 for species A and treatment 4 vs. 5 for B). See Underwood (1986) for more details on designing field experiments to test interspecific competition.

	Treatment				
Species	1	2	3	4	5
A	20	10	10		
B			10	10	20

A second criticism is that the few field studies that are based on experimental manipulations generally lack controls and replication of the basic experimental units (i.e. pseudoreplication sensu Hurlbert 1984). For example, in studies of population response to removal of a competitor experimental units are stream sections of sympatry and allopatry, which must be dispersed in space and measured through time if broad inferences about competition are to be valid. Thus, the shifts Fausch and White (1981) observed in positions of brook trout after removal of brown trout from a Michigan stream might have been due to the effect of electrofishing, for which there was no control, or changes in environmental factors during the study, which could not be assessed because sympatry and allopatry were not replicated.

A third problem arises when studies of interspecific competition conducted in laboratory streams are used to predict which species will dominate in nature. Because these artificial settings simulate natural environments to an unknown degree, inductive inferences about the effects of competition may be tenuous when applied to natural streams. For instance, Fausch and White (1986) reported on an investigation of competition among juvenile brook trout, brown trout, and coho salmon in a laboratory stream, although they made only tentative inferences to Great Lakes tributaries.

Despite these problems, one cannot deny that these studies are valuable in generating relevant hypotheses about competition among stream salmonids and in helping elucidate important mechanisms, which experiments that simply measure population response cannot do (Schoener 1974, 1983). Diamond (1986) presented an excellent discussion of the interplay among field experiments, laboratory experiments, and two types of natural experiments (i.e. what I term empirical evidence) and recommended a pluralistic approach using all these methods to discover important mechanisms controlling communities. Nevertheless, the studies I criticize here are not rigorous tests of interspecific competition in the field. Indeed, the recent furor in the literature of community ecology over the proper design of field experiments to test competition and predation has led to a number of reviews on this subject (Connell 1983; Schoener 1983; Diamond 1986; Underwood 1986). For instance, Underwood (1986), a marine intertidal ecologist, proposed several experimental designs to sort out the effects of interspecific versus intraspecific competition for two species (Table 2). The problem is that even his simplest design, which calls for two densities of allopatry for each species and one of sympatry, may

be unmanageable for field experiments on stream fishes because each of the five treatments must also be replicated.

Where does this leave us in trying to solve the practical problem of predicting effects of introduced salmonids in streams? Because salmonids are one of the most studied groups of fishes, one would expect there to be sufficient evidence to permit predictions for at least some pairs of species. To address this problem, I chose to consider one pair of salmonids, brook and rainbow trout, because there exists a relatively large body of empirical and experimental evidence related to the suspected competition between these two species in streams.

Brook versus Rainbow Trout: A Case Study

Rainbow trout are thought to be superior competitors over brook trout in North American streams. The two species are sympatric throughout the interior of the western United States, where neither are native, and in the northeastern United States and Appalachian Mountains, where brook trout are native (MacCrimmon and Campbell 1969; MacCrimmon 1971). It is interesting that brook trout are not common in lotic systems of the Pacific coast region and inland where steelhead or redband trout (sensu Behnke 1979) are indigenous despite widespread introduction (Moyle 1976; R. J. Behnke, pers. comm.), perhaps due to the competitive ability or superior adaptation of the native fish.

Empirical Evidence

Sympatric populations of brook and rainbow trout have been intensively studied in two locations, Sagehen Creek, California (Gard and Seegrist 1972; Seegrist and Gard 1972; Gard and Flittner 1974; Erman 1986), and the Great Smoky Mountains National Park in Tennessee and North Carolina (Moore et al. 1983, 1986; Larson and Moore 1985). These species show similar patterns of distribution along elevation gradients in the two regions. Brook trout are found in allopatry in the upstream, higher gradient, lower temperature reaches, often extending nearly to the headwaters. Proceeding downstream, a zone of sympatry occurs, after which rainbow trout are found in allopatry in the downstream, lower gradient, higher temperature reaches. In both locations, brown trout also occur farther downstream.

In Sagehen Creek during 1952–61, brook trout were most abundant in three upstream sections with 3–7% gradient and lower temperatures, but populations extended downstream through seven other sections in a long zone of sympatry with rainbow trout (Gard and Flittner 1974). Gard and Flittner (1974) attributed the upstream limit of brook trout distribution to the lack of pool habitat during fall and winter low flows because this species later thrived in small man-made pools in a meadow farther upstream. In contrast, they proposed that the upstream distributions of rainbow and brown trout were limited by low water temperature.

Fluctuations in beaver (*Castor canadensis*) populations play an important role in shaping salmonid communities in Sagehen Creek because it is thought that the pool habitat they create behind their dams favors brook and brown trout (Gard 1961; Gard and Seegrist 1972). For instance, brook and brown trout populations declined in the downstream reach after beaver dams washed out in a December 1955 flood. Many of the dams were not rebuilt, probably because nearby aspen (*Populus tremuloides*) was depleted. Partly as a result, numbers of brook and brown trout in the stream as a whole declined significantly ($P < 0.05$) during the 10-yr period whereas rainbow trout abundance remained stable (Gard and Flittner 1974).

In a related paper, Seegrist and Gard (1972) proposed that the timing of winter and spring floods was also critical in shaping these sympatric populations because of differential effects on young-of-the-year (YOY) survival for spring versus fall spawners. Severe winter floods were thought to decrease year classes of fall-spawning brook trout by washing away eggs and fry, allowing fry of spring spawning rainbow trout to thrive the next year due to decreased interspecific competition. Spring and summer floods appeared to have similar detrimental effects on rainbow trout YOY, thus favoring brook trout. Although the evidence for these patterns is somewhat equivocal for Sagehen Creek, Nehring (1987) found that magnitude of flow during and soon after emergence was inversely related to, and had similar differential effects on, recruitment of brown and rainbow trout over 4–8 yr in each of 12 Colorado rivers. Schlosser (1985) also found that the timing of spring and summer floods in a warmwater Illinois stream differentially reduced recruitment of certain species, which strongly affected the fish community.

Erman (1986) sampled fish populations in Sagehen Creek during 1982–83 in the same sections used in earlier studies and compared the data to determine whether the distribution of brook and rainbow trout had changed markedly during the 31-yr period. He found that brook trout had disappeared from three sections farthest downstream and the most upstream section and were significantly less abundant at the second and third sections from the upstream end where they had previously been most abundant. He attributed the decline upstream to severe droughts in 1976–77 and a severe winter flood in 1982, but observed that brook trout had recolonized the headwaters by 1985. In contrast with brook trout, the distribution of rainbow trout was generally similar to that found earlier. Confounding factors were that fingerling brook trout were stocked in the downstream reach until 1951, although Gard and Seegrist (1972) proposed that few of these fish survived over winter, and that rainbow trout introduced in a downstream reservoir completed in 1969 probably migrated upstream to spawn (Erman 1986). Overall, it appears that floods and droughts, and changes in habitat due to fluctuating beaver populations, have had marked effects on the distributions of brook and rainbow trout in Sagehen Creek (Gard and Seegrist 1972; D. C. Erman, Department of Forestry and Resource Management, University of California, Berkeley, CA 94720, pers. comm.) which may have been sufficient to mask the effects of interspecific competition.

Larson and Moore (1985) presented clear evidence that the distribution of brook trout declined markedly in Great Smoky Mountains National Park (GSMNP) concurrent with expanded rainbow trout distribution. The length of stream occupied solely by brook trout declined 60% (64 km versus 158 km) during about 40 yr, based on surveys made in 1935–36 and 1972–77, whereas rainbow trout invaded 94 of 158 km of stream surveyed.

This distribution is also related to stream gradient. In the early 1900's, allopatric brook trout were found in streams with less than 5% gradient, but now occur only in higher altitude headwater streams of more than 15% gradient (see fig. 1 in Larson and Moore 1985). In GSMNP, most stream sections with sympatric populations are reported to have 12–13% gradient, which is steep, and most zones of sympatry are less than 3.2 km long. It is interesting that the sympatric zone in Sagehen

Creek shrank from about 10.5 km during 1952–61 (Gard and Flittner 1974) to about 3.2 km in 1982 (Erman 1986).

Larson and Moore (1985) also found negative relationships between densities of adult brook and rainbow trout and between adults of either species and YOY of the other species. This well-documented encroachment is consistent with the hypothesis that rainbow trout exclude brook trout from preferred habitat by competition. However, predation, habitat degradation due primarily to earlier logging, and fishing cannot be ruled out as contributing factors, although eliminating logging and curtailing illegal fishing in GSMNP has not halted the rainbow trout advance. The importance of predation on young by adults of either species has yet to be tested in streams, as far as I know, although Alexander (1977, 1979) found that brown trout ate many more brook trout YOY than their own young in a Michigan stream.

Judging from this body of empirical evidence, gradient and temperature are suspected to be important factors affecting the relative competitive abilities of brook and rainbow trout. An apparent paradox is that although brook trout occur in higher gradient headwater reaches when the species are sympatric, they use lower velocity microhabitat than rainbow trout when either sympatric or allopatric (Cunjak and Green 1983). Regardless of the habitat, brook trout appear to prefer low-velocity positions in pools adjacent to cover (Butler and Hawthorne 1968; Cunjak and Green 1983) whereas rainbow trout prefer higher velocity positions without cover in riffles and runs (Hartman 1965; Smith and Li 1983). This difference in microhabitat use, coupled with differences in their innate temperature tolerances (cf. Coutant 1977; Peterson et al. 1979; Power 1980), should prove important in determining the outcome of competition in different environments. For instance, Larson and Moore (1985) reported no encroachment by rainbow trout in a higher elevation but lower gradient brook trout stream with no migration barriers, although supporting data on temperature and fish populations have yet to be measured (S. E. Moore, Great Smoky Mountains National Park, Gatlinburg, TN 37738, pers. comm.).

The larger body size and innate competitive ability of rainbow trout may also play a role. For example, rainbow trout body weight is greater than brook trout from age 1 + in southern Appalachian streams (Whitworth and Strange 1983; Larson and Moore 1985). Rose (1986) proposed that YOY rainbow trout suppressed growth of YOY brook trout in a Lake Superior tributary during the first months of life by superior exploitative and interference competition for invertebrate drift, but did not conduct manipulative experiments to support the conclusions.

Experimental Evidence

Various investigators have studied interactions between brook and rainbow trout in both the field and laboratory. The strongest evidence that rainbow trout eliminate brook trout by competition and/or predation is that brook trout standing crops increase after rainbow trout are removed. Moore et al. (1983, 1986) removed rainbow trout from four GSMNP streams that had sympatric populations by electrofishing each year for 6 yr (1976–81), which reduced rainbow trout density by 89% on average to less than one adult per 100 m^2 and eliminated YOY recruitment. Recent work indicates that rainbow trout were subsequently eliminated from reaches of two streams (S. E. Moore, pers. comm.). Brook trout populations increased in all streams after rainbow trout were removed, although YOY brook trout increased only during the last year of the study. In contrast, populations of adult brook trout in three allopatric control sections showed no consistent trends during the same period, although YOY abundance increased markedly during 1981 as for the sympatric sections suggesting that widespread recruitment success may be primarily controlled by abiotic factors.

This research is one of the best field studies of interspecific interactions among stream fishes of which I am aware, although I suspect that it was planned primarily as a management effort to forestall the upstream advance of rainbow trout. It stands out because it included replicated experimental manipulations and control populations in many sections of several streams. For example, Moore et al. (1986) sampled 17 300-m sections in five streams during each year of the 6-yr period. With this same effort, they could have employed Underwood's (1986) experimental design (Table 2) and had three replicates of each treatment (e.g. they had four replicates of brook trout allopatry). Other reports (Moore et al. 1983; Larson and Moore 1985) indicate that they also electrofished many more sections, all of which indicates to me that sound experimental field tests of interspecific competition among stream fishes are not impossible.

Studies such as these documenting increased numbers and biomass of the subordinate species after removal of the suspected dominant usually require years to record the full population response for fishes and are generally not useful in elucidating the mechanisms of interaction (Schoener 1974, 1983). For instance, Larson and Moore (1985) observed that brook trout used positions adjacent to main currents when in allopatry, but shifted to unfavorable positions along the shallow margins of GSMNP streams where rainbow trout were abundant. This pattern suggests a niche shift due to interspecific competition, but the authors collected no data to substantiate their report. Thus, other investigators have opted for measuring the behavioral ecology of these species in the field or laboratory in hopes of elucidating proximate competitive mechanisms on a shorter time scale.

The purpose of such behavioral studies is to determine whether rainbow trout can exclude brook trout from favorable stream positions (cf. Fausch 1984) by interference competition via agonistic behavior. Newman (1956) conducted the first study of competition between brook and rainbow trout in laboratory aquaria and in the field, using an observation tank sunk in a pool of Sagehen Creek for the latter. His laboratory experiments were relatively unsophisticated, but he recognized the problems of artificial environments and feeding regimes and the effects of temperature and size advantages on the outcome of competition. In Sagehen Creek, he could consistently observe only four rainbow and two brook trout of different sizes, so he could draw no firm conclusions other than that body size appeared very important in determining dominance for optimum positions in the stream.

Cunjak and Green (1983, 1984, 1986) published three papers on habitat use, dominance, and the effects of temperature on dominance of rainbow and brook trout, but in my opinion, some results are misinterpreted and others are equivocal due to problems with experimental methods or incomplete documentation. Cunjak and Green (1983) measured microhabitat use of the two species by underwater observation in two Newfoundland streams, one with allopatric brook trout and the other with the species sympatric. They found that brook trout held positions in low water velocities adjacent to cover in both streams whereas rainbow trout in sympatry held positions in higher water veloc-

ities farther from cover. The differences between positions of the species in sympatry were significant ($P < 0.01$), from which they concluded that rainbow and brook trout have microhabitat preferences that allow them to coexist. They further suggested that neither species was dominant to the extent that the subordinate was excluded to marginal habitats, but that the species were partitioning microhabitat due to interactive segregation (sensu Brian 1956; Nilsson 1967).

I believe that Cunjak and Green (1983) misinterpreted the meaning of their results. Interactive segregation refers to niche shifts by one or both species that result from interspecific competition and serve to minimize overlap in resource use. To test for such niche shifts that allow coexistence would have required designed experimental manipulations. For instance, part of such an experiment might involve removing brook trout from sympatry to determine whether rainbow trout shift to more favorable stream positions, grow faster, or have higher survival in response. In contrast, the evidence that Cunjak and Green (1983) presented suggests that under these conditions the species may be preadapted to use different microhabitats and therefore show little overlap in their fundamental niches, in which case the resource partitioning would be due to selective segregation instead. Selective segregation refers to genetically fixed differences in resource use that preclude interaction (Nilsson 1967). However, rather than use these relatively imprecise terms, I believe discussion should focus on the degree of niche shift caused by interspecific competition, as measured by changes in behavior, physiology, growth, survival, or fitness.

In a companion study, Cunjak and Green (1984) pitted pairs of rainbow and brook trout against one another in two enclosures positioned on either side of an observation tank in a natural stream. For each 10-d trial, one pair was enclosed in a lower velocity location (1–7 cm/s) and one in higher velocity (8–35 cm/s). Mean water temperatures during the trial ranged from 10 to 20°C. Dominant fish were judged to be those that initiated the most aggression and had priority in position choice whereas subordinates showed darker coloration. However, a problem is that in the high-velocity trials the lengths of fish were poorly matched, so that for five of eight pairs the eventual dominant fish was initially larger by 9–22 mm for fish of 110–172 mm fork length (see their table 1). For the other three trials, lengths were within 2 mm, which was reflected in the results — rainbow dominated twice and brook trout once. Unfortunately, the paired *t*-tests used by Cunjak and Green (1984) to claim no significant difference of initial lengths and weights only assess the mean difference among pairs of fish and can mask large, biologically significant differences that affect dominance between individual pairs. Size generally confers dominance within species of salmonids (Noakes 1980) and also has important effects on competition between species (e.g. Fausch and White 1986). Thus, I judge that the results were equivocal for the higher velocity microhabitat, which was also not very swift relative to velocities measured in high-gradient streams nor, as the authors stated, compared with velocities they measured at brook trout positions in the field (Cunjak and Green 1983).

In low-velocity trials, lengths were more evenly matched, and brook trout dominated in five of six trials even though they were of equal length or shorter by up to 10 mm in four of the five. Therefore, the evidence presented in these two studies supports only the conclusions that brook trout hold positions in lower velocities and closer to cover than rainbow trout in a natural stream and appear to dominate rainbow trout in low velocities when confined in one-on-one trials.

Cunjak and Green (1986) also wanted to know how competition between brook and rainbow trout changes with temperature, so they pitted pairs of juvenile fish against one another in aquaria at 8, 13, and 19°C. They assumed that 13°C was optimal for brook trout and 19°C optimal for rainbow trout, based on the literature. They fed the fish a pelleted diet, determined dominance as before (Cunjak and Green 1984), and measured weight change and behavior for 10 d (R. A. Cunjak, Department of Fisheries and Oceans, P.O. Box 5030, Moncton, N.B. E1C 9B6, pers. comm.). They found that brook trout grew better than rainbow trout at 8 and 13°C, but lost weight at 19°C. Rainbow trout always lost weight, but declined least at 19°C. Differences in growth between the species were significant ($P < 0.05$) only at 8°C.

Brook trout were dominant in most trials where dominance was established at 8°C (four of five trials) and 13°C (five of six trials). Brook trout also established dominance sooner at 13°C than at other temperatures ($P < 0.05$) and showed significantly more agonistic behavior than rainbow trout at 13°C ($P < 0.05$). Based on this evidence, Cunjak and Green (1986) concluded that brook trout dominated rainbow trout at colder temperatures, especially 13°C, but that neither species had a definite competitive advantage at 19°C.

My criticism of their work is that paired *t*-tests were again used to claim no difference in initial lengths and weights, but the data were not presented, so one cannot determine whether dominance was an artifact of differences in body size. Some fish died (see sample sizes in their tables 1 and 2), apparently on the last day of experiments (R. A. Cunjak, pers. comm.), but this was not reported. Moreover, the authors suggested that results may have been different had they used a flowing environment with natural drifting invertebrates as food, but the logistics of running several experiments simultaneously prevented them from using the stream enclosures from their previous study (R. A. Cunjak, pers. comm.).

Conclusions

The upshot of this review is that, in my view, studies of competition between introduced and native salmonids, both in the field and laboratory, have rarely been careful enough to elucidate the critical mechanisms involved. For example, the important questions that emerge from the body of empirical and experimental evidence presented above about how water velocity and temperature interact to affect competition between rainbow and brook trout remain largely unanswered. Do rainbow trout dominate in high-velocity microhabitat, and does this dominance increase with temperature? Clearly, this needs to be studied for YOY, juvenile, and adult fish, preferably through a combination of appropriate field and laboratory experiments.

But how should experiments be designed to avoid the pitfalls of the past? The central idea is to test the strength of interspecific competition versus that of intraspecific competition by manipulating densities of the two competitors in replicate stream sections (see Table 2 and Connell 1983). In other words, one must ask whether adding or removing the competing species has a greater effect than adding or removing the same number of intraspecific competitors. Designs based on this principle (see Underwood 1986) also obviate problems caused when the overall densities are changed by adding or removing only interspecific competitors.

Replication of each treatment in different streams and, ideally, over several years is necessary to preclude the possibility that the results are an artifact of an unusual occurrence, a problem Hurlbert (1984) termed "demonic intrusion." Beyond this, more replication increases the precision and generality of the results (sensu Levins 1966). But although replication of treatments can be easily done when studying interactions among sessile marine invertebrates on small plots of intertidal zone (e.g. see Underwood 1986), the size and mobility of stream salmonids requires that populations in entire stream sections be manipulated. In addition, one must design appropriate controls to account for the effects of electrofishing, enclosing sections with weirs, or other perturbations used to establish the treatments. Moore et al. (1983, 1986) demonstrated, however, that such experiments are not an impossible undertaking in small streams (see previous section).

Another important factor in experimental design is choosing the scale of response variable used to detect competition. Ideally, one would always measure changes in fitness, but this requires careful monitoring of reproduction and survival over several generations, which is generally prohibitive for fish. Comparing survival of different age classes in sympatry versus allopatry for several years would be the next best approach, but most investigators are constrained by logistics to measuring changes in growth, physiology, or behavior (e.g. Fausch and White 1981, 1986) which can be achieved in a time scale ranging from days to months. A further confounding factor to address for any time scale is the effect of competition on immigration or emigration of fish.

While I have primarily highlighted field experiments, laboratory experiments require many analogous design considerations. In addition, sufficient acclimation of organisms to laboratory apparatus and feeding regimes is of paramount importance (cf. Fausch 1984). A final problem that must be considered in all experiments is the effect of fish size. As emphasized previously, size may have overriding effects on interspecific dominance. In laboratory tests, fish should be of nearly equal size (e.g. Fausch and White 1986) or of some predetermined size difference. The size structure of fish populations also has important effects on measuring competition in the field (e.g. Fausch and White 1981; Werner 1984) and must be accounted for there, although it is less easily manipulated. Finally, investigators should not overlook the advantages of combining the results of experiments with empirical evidence in a pluralistic approach (cf. Diamond 1986) to assessing the effects of competing salmonids.

Returning to the question posed above, if appropriate experiments indicate that rainbow trout do dominate brook trout in high-velocity microhabitat, and that this dominance increases at warmer temperatures, it may explain why rainbow trout exclude brook trout from progressively higher gradient reaches of both western (Gard and Flittner 1974; Erman 1986) and eastern United States mountain streams (Moore et al. 1983, 1986; Larson and Moore 1985), especially at lower altitudes where mean summer water temperatures exceed 15°C. Conversely, we also need to know if brook trout of various ages dominate in lower velocity microhabitats and whether this dominance increases at lower temperatures. If this is true, it may explain why brook trout continue to maintain populations in the headwaters of Sagehen Creek and GSMNP streams in the face of rainbow trout invasion, especially in low-gradient, high-altitude reaches, as Larson and Moore (1985) suggested.

Clearly, even elucidating these mechanisms will likely not ensure perfect predictions. Competitive superiority may fluctuate in natural environments due to the differential effects of floods and droughts (Moyle and Vondracek 1985; Erman 1986), predation or fishing (Fausch and White 1981), or due to shifting availability of beaver pond habitat (Gard and Seegrist 1972; D. C. Erman, pers. comm.). Moreover, superior adaptation of the indigenous species to a local suite of environmental factors, predators, and parasites may render an invader impotent (cf. Marnell et al. 1987), although this has apparently not been the case for rainbow trout in the GSMNP where brook trout are native. However, combining knowledge about the interacting effects of temperature and water velocity on competition between brook and rainbow trout with measurement or control of these other factors will allow us to begin to predict the outcomes of introductions of either species on the other in specific environments.

Although one of my main purposes here was to review literature on competition between brook trout and rainbow trout, I believe that the empirical and experimental evidence about competition between other species of salmonids in streams is fraught with similar problems. Moreover, although fish ecologists studying salmonid competition may understand the limitations of our work, fisheries biologists and laymen who apply these results to management may accept them rather uncritically. Clearly, more careful tests of competition and predation are needed for important sets of species, using both laboratory and field experiments, if we are to reach the stage where we can predict the effects of introduced salmonids on native ones in streams.

Acknowledgments

The ideas in this paper were first presented at a symposium on introduced species in natural systems at the American Society of Ichthyologists and Herpetologists' annual meeting in Albany, NY, June 1987. I thank Peter Moyle for inviting me to present that paper and encouraging me to write this manuscript. Bob Behnke, Rick Cunjak, Peter Moyle, Don Erman, Steve Riley, Steve Moore, John Gibson, and Gary Grossman made insightful comments on an earlier draft, and Geoff Power and Fred Whoriskey provided constructive reviews. I am grateful to Bob Behnke for many helpful leads and for frequent critical discussions that shaped these ideas.

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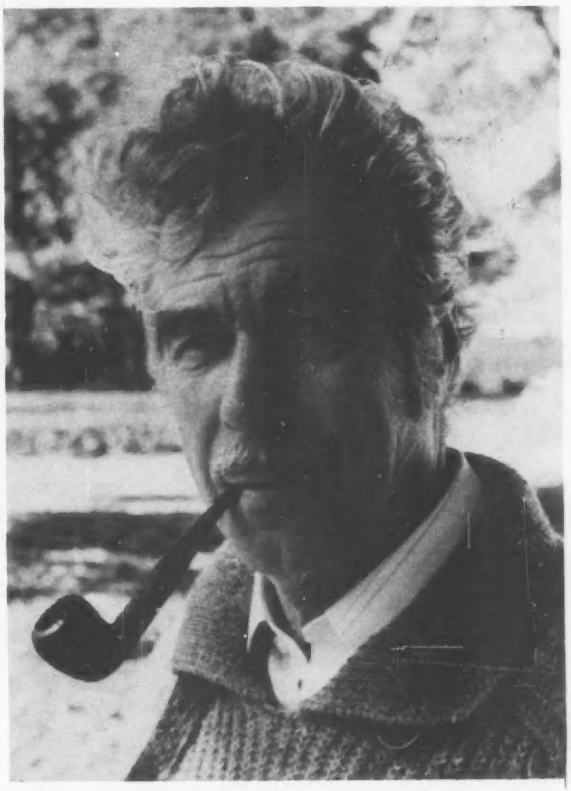
OBITUARY/NÉCROLOGIE

Kenneth Howard Loftus

1923–1988



Kenneth H. Loftus, a fisheries ecologist, manager, and leader *nonpareil* in his chosen field, died on Sunday April 10, 1988, at Richmond Hill, Ontario, following a lengthy bout with cancer. His youthful vigour and thoughtful, caring enthusiasm for the well-being of our aquatic heritage animated him to the very end.

Although formally retired, Ken actively pursued a consulting career to the time of his death, despite the increasing physical discomfort in doing so. His outward comportment during this final trial was such that only his closest colleagues were aware of the debilitating burden under which he laboured for so long. His passing marks the loss of one of those few who truly made a difference in the way that decisions were made, and will continue to be made, concerning the preservation of our aquatic environment. His legacy, in the form of both published works and colleagues persuaded by his views, will long survive him.

Ken's early days were spent on the family farm near Burford, in southwestern Ontario; doubtless it was that venue which formed both his esteem for nature (he had a life-long appreciation for Aldo Leopold's writings), and his penchant for hard work, an attribute that characterized his life.

Ken's formal fisheries training began at McMaster University in Hamilton, Ontario, where he earned his Bachelor of Arts degree in 1945. He received graduate training at the University of Toronto, which culminated in a Master's degree in 1948. It was there that Ken came under the tutelage of Professor F. E. J. Fry, and subsequently developed a relationship that was to serve Ontario fisheries well for the next 40 years. It was also at the University of Toronto that Ken developed close ties with the late N.S. Baldwin, who was later to become the Supervisor of Fisheries Research in Ontario, and the late N. V. Martin, who would be the Senior Scientist in charge of lake trout research for the Province. This triumvirate harboured much of the fisheries knowledge within the Ontario government in the years following the creation of the Department of Lands and Forests in 1947, which had a new Fisheries Division under the direction of the late W. J. K. Harkness.

Following a brief period at main office in Toronto, where he served as biologist-at-large, Ken accepted a position in 1948 as management biologist with the new department at Sault Ste. Marie. He realized very early in his career an observation that was to influence his subsequent actions, that research had not provided all the necessary information he needed to do his management job effectively. His principal contribution at the time was to mount a collaborative effort with other management and research colleagues, initiating a study that resulted in the seminal paper on river-spawning populations of lake trout in Lake Superior, prior to their near-extinction by the exotic sea lamprey (Loftus 1958). This was merely the earliest of his numerous attempts to illuminate management practices with the information to be garnered from informed research programs.

In 1957, Ken was promoted to Assistant District Forester at Sioux Lookout, but declined the position in favour of a Fisheries Research Supervisor's opening at Maple, Ontario, a position that had been recently vacated by N. S. Baldwin. Ken viewed this position as a challenge that would enable him to make fisheries research better understood by the management component of the Department. At that time, fisheries research was viewed as a low-priority extravagance that was merely tolerated, but not strongly promoted within existing government policies. Ken's approach to this less than supportive stance taken by many of the government administrators was to initiate a slow, stepwise growth of the Fisheries Research Section by recruiting some of the best scientists available at that time within the fisheries discipline. At the same time, he ensured that the technical language and concepts of the research scientists were adequately translated into practical management application, and that administrators were fully cognizant of the valuable role that fisheries research could play in the future management of Ontario's fisheries.

In 1971, Ken teamed up with H. A. Regier to sponsor the "Salmonid Communities in Oligotrophic Lakes Symposium" (SCOL) held at Geneva Park, Ontario. This landmark symposium was a breakthrough in both innovation and creative thinking. Far from being a traditional "show and tell" symposium, SCOL was a product of careful preplanning to ensure that the appropriate mix of research scientists and fisheries administrators from both Europe and North America attended, and interacted in a meaningful way. This approach guaranteed that the ultimate product of the symposium would be suitable for future management application, and would provide essential information to fisheries managers working at the leading edge of their discipline. The subsequent volume of the *Journal of the Fisheries Research Board of Canada* emanating from this symposium (Loftus and Regier 1972) was a boon to fisheries managers working within the north-temperate zone because of its novel and effective approaches to traditional problems.

Ken ensured that the lessons learned in this symposium were passed on to every management biologist in the Province. To this end, he organized creative training sessions in which the symposium was reenacted in detail, the principal players in the latter instance being not the research scientists who filled the original roles, but rather the management biologists who would apply the new science. This successful format, established by Ken in the early 1970's, has persisted through several interactive symposia sponsored by Ontario over two decades to the present day. It is generally regarded as the quintessential formula for the transfer of science in fisheries to the managers who need to apply it.

In 1972, Ken was elevated to the position of Director of the Sport Fisheries Branch in main office at Toronto. While he regretted the move from research, he recognized the significance of his new position and the opportunity it would provide for his continued efforts to promote science transfer, not only to the fisheries managers but also to the users of the resources, the public. Ken continued in his sport fisheries' role until 1976, at which time he became Director of the Fisheries Branch, the highest-ranking fisheries position within the Province. Again, Ken regarded this position as an opportunity to further his objectives of science transfer. His strong promotion of the "Strategic Planning for Ontario Fisheries" (SPOF) initiative established another high plateau for Ontario fisheries and potentially, federal–provincial collaboration. Despite his most valiant efforts, he was met head-on with frustration after frustration in the attempted implementation of the SPOF package as part of a comprehensive Ontario government policy on fisheries. Notwithstanding the repeated failure for complete SPOF acceptance and implementation, certain spin-offs could be considered as landmark victories. For example, a network of fisheries assessment units manned by fisheries biologists was established across the Province. These fulfilled a critical hiatus that existed within the previous management system. Additionally, an Ontario resident angling licence was implemented, with the guarantee from government that revenues derived from their sale would be reinvested into fisheries programs. Most recently, the SPOF concept has been reevaluated with consideration to be given in the near future to full implementation. While many people have contributed to the concepts and objectives of SPOF, it will always be remembered as a major legacy of Ken Loftus.

In 1980, Ken left the prestigious position of Director of the Fisheries Branch into the less onerous but equally prestigious one of Provincial Fisheries Scientist. While the new position was less demanding of his time, it was important, nonetheless, and Ken's strong input to fisheries initiatives continued at his previous pace.

Ken retired from the Ontario Ministry of Natural Resources in 1984, but he was not a willing retiree — nor did he ever retire from his fisheries interests. His consulting and volunteer attendance at fisheries meetings kept him abreast of fisheries matters right up to the time of his death.

Ken's career with the Ontario Department of Lands and Forests (which became the Ontario Ministry of Natural Resources in 1973) was closely paralleled by his other fisheries interests. He was an early participant in the Great Lakes Fishery Commission's (GLFC) concerns with the sea lamprey problem on the Great Lakes, and in 1957 was appointed to the Scientific Advisory Committee of the Commission, a position he occupied until 1972. He remained active throughout the remainder of his career in binational fisheries issues on the Great Lakes. Ken served on the Water Quality Board of the International Joint Commission (IJC) from 1974 to 1976, and it was sometime during this period that he became aware of the need to have a combined binational effort between the two commissions (GLFC and IJC), jointly charged with resource and environmental responsibilities, respectively.

In 1973, Ken became a commissioner for GLFC and, acting in that capacity, he was a strong proponent for strategic planning for fisheries management in the Great Lakes. His forceful efforts to retain native fish stocks in the Great Lakes against increasing opposition from the "salmon-planters" resulted in an official position taken by GLFC on lake trout rehabilitation. This position stated that one of the major responsibilities of GLFC was to ensure a rehabilitation of self-sustaining lake trout stocks, and "...that stocking with hatchery-reared lake trout is an essential step toward achieving self-sustaining lake trout populations... a major Commission objective" (GLFC 1976). This ecologically sound position, unfortunately, has eroded somewhat in recent years, but those who understand the importance of such a strong stance continue to support it.

Ken chaired the "Fish Commission," as it is commonly referred to, for approximately six years, and his egalitarian approach in this role made him friends in all constituencies. A Canadian chairman dealing with two federal governments, eight states, and one province, of necessity must execute a diplomatic balancing act. To do this impartially but effectively, without sacrificing one's own principles, or without garnering favour for one's jurisdiction, must seem to be a next-to-impossible task. Ken's strength of character provided him the advantage of being firm but fair. He was well respected by friend and opponent alike, right up to the time he retired from the Commission in 1984.

As a fisheries administrator, Ken was never surrounded by employees — but rather by friends. His demeanour was such that he could be firm and forceful without upsetting sensitive personalities. Despite his friendly repartee, he was surrounded by an aura that permeated a business meeting, and no one ever had any doubt about who was in charge.

Surprisingly, however, he had some personal doubts about his own scientific capabilities. He often berated himself in the presence of others about his inability to do good research, despite the fact that he had never really had much opportunity to do so because of his demanding administrative responsibilities. Yet he had a scientific understanding of the significance of the research of others that was truly profound. His comprehension about another scientist's research was often more insightful from a management viewpoint than that of the sci-

entist who had done the research. Ken's broad understanding of management problems and his intimate knowledge of the publics' wants and needs allowed him to rapidly place the conclusions of fisheries research in appropriate perspective to management requirements.

Despite the fact that Ken but rarely afforded himself the luxury of personal research, his list of refereed, primary journal publications is impressive. In 1975, while under contract to the Fisheries Research Board of Canada, Ken served on the Fisheries Resources Committee. His specific charge was to determine the status of Canada's fisheries in the inland waters and on both coasts, determine the extent of man's interventions, and recommend the science needed for future rehabilitation — an enormous charge. Ken accepted the challenge, travelled across Canada from coast to coast, interviewing fisheries scientists and others who could provide pertinent fisheries knowledge, and synthesized all of the information into a comprehensive report (Loftus 1976). Today, some 12 years later, Ken's report on "Science for Canada's Fisheries Rehabilitation Needs" is as relevant as the day it was written, and represents a landmark document in the long history of Canada's fisheries.

Ken always retained a personal, Socratic philosophy that was both homespun and sincere. His philosophical approach to most fisheries problems inevitably resulted from many hours of intensive thinking, followed by exhaustive exploration with friends and colleagues. These explorations in depth were a valued exposition to his thinking by those who knew him well. There was hardly an area in fisheries management that had not been given serious scrutiny by Ken at one time or another, and his colleagues never ceased to draw upon this font of knowledge.

To use a term that is sometimes overworked in the media these days, Ken was a leader possessed of great charisma. The main substance of his charisma lay in the certain knowledge that he would act to the best of his ability to do the "right thing," as opposed to the popular or expedient thing. There are occasions when this intellectual honesty sometimes confused his opponents of the moment, as it was his style not to act upon an idea unless he had tested it in extensive debate with colleagues whose judgement he respected. Despite his great powers of reasoning and the ability to place complex questions in appropriate perspective, Ken retained a sincere and disarming modesty that projected a quiet confidence.

Many of Ken's personal philosophies are accepted as ecological dogma in the fisheries discipline today. Others, though equally sound, are still subject to debate. He was adamant that science transfer from researcher to manager, and eventually to the public, should be a primary consideration of all fisheries managers. Throughout his career he strove mightily to achieve this goal. Ken believed that natural fish communities under exploitation should be afforded an appreciable degree of protection, even down to the phyletic level of stocks, as he was a firm believer in the potential adaptability built into the genetic structures of fishes. This belief was highly unpopular in some quarters, coming as it did in the midst of a Pacific salmon planting boom in the Great Lakes during the 1970's and 1980's. Along this same vein, Ken saw the need to rehabilitate natural stocks of fishes rather than to "enhance" them; the latter term he saw as fraught with the danger of misinterpretation. He also believed that the proponents of salmon planting in the Great Lakes were creating a need for people's expectations where one had not existed previously, thus burdening the already overly laden management system. Most of Ken's insightful prognostications in these matters are becoming fact.

Throughout his lifelong dedication to his work, Ken's pride in home and family was apparent to those who knew him best. He is survived by his wife, one daughter, three sons, and seven grandchildren. Vacations at the Baptiste Lake cottage in eastern Ontario were a family event that were eagerly anticipated by all. It was there that Ken allowed himself but one near self-indulgence. It was an annual gathering of his friends and colleagues at his Baptiste Lake cottage that coincided with the opening of deer season. These gatherings were most inappropriately called "hunt camp," but hunting had very little to do with the activities there, although a deer was occasionally shot, perhaps about once a decade. Ken revelled in these gatherings which were festschrifts for some, fisheries gab sessions for others, followed each evening by high-stakes (five or ten cents) poker games. At these times, Ken could often be found firmly ensconced in front of a megalithic fireplace that he had constructed, espousing his latest philosophy to willing ears crowded about. These sessions at Baptiste Lake (and previously, Lobster Lake) were memorable occasions that have continued for more than 25 years. It was there that much of Ontario's fishery policy was formulated!

One cannot conceive of Ken Loftus without considering the legacy left at his passing. At the fundamental level, these assumed the form of basic Socratic interactions with colleagues, and Herculean physical structures. Ken enjoyed building things with his hands, and the fireplaces and stone walls at both Richmond Hill and Baptiste Lake rival the massive structures left by the megalith builders. Indeed, Ken's stone structures will persist, much like his philosophies, because they are sturdy, well-planned, and resistant to erosion.

Ken Loftus has left a very large mark on the face of the globe. Numerous people who enjoy our rich legacy of aquatic resources in North America will never recognize the extent of their debt.

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ANNOUNCEMENTS/AVIS

International Conference
Biomanipulation, Tool for Water Management

Artis Zoo, Amsterdam, The Netherlands
August 8–11, 1989

From 8 to 11 August 1989, the Royal Netherlands Academy of Arts and Sciences and the Institute for Inland Water Management and Waste Water Treatment will organize an international conference entitled "Biomanipulation, Tool for Water Management" (Artis Zoo, Amsterdam, The Netherlands).

Biomanipulation defined as "the deliberate exploitation of the interactions between the components of the aquatic ecosystem in order to reduce the algal biomass" receives more and more attention as a tool to study and manage ecosystems. The aim of the conference is to enhance the discussion between aquatic ecologists studying trophic interactions and people working in the field of water and fish management. The conference will focus on the possibilities of biomanipulation. The program covers biomanipulation research in laboratory, enclosure systems, and lakes as well as modelling studies.

Requests for further information should be addressed to Convention Bureau van Namen en Westerlaken, P.O. Box 1558, 6501 BN Nijmegen, The Netherlands; telephone: 31 80 234471.

Conférence internationale
La Biomanipulation, outil de gestion des eaux

Zoo Artis, Amsterdam, Pays-Bas
8–11 août 1989

Du 8 au 11 août 1989, l'Académie royale des arts et des sciences des Pays-Bas et l'Institut pour la gestion des eaux intérieures et du traitement des eaux usées prévoient une conférence internationale intitulée « La biomanipulation, un outil pour la gestion des eaux » (Zoo Artis, Amsterdam, Pays-Bas).

La biomanipulation, définie comme « l'exploitation délibérée des interactions entre les composants d'un écosystème aquatique afin de réduire la biomasse algale », reçoit de plus en plus d'attention comme outil d'étude et de gestion des écosystèmes. Le but de la conférence est de favoriser les discussions entre les écologistes du milieu aquatique étudiant les interactions trophiques et les personnes travaillant dans le domaine de la gestion des eaux et du poisson. La conférence portera sur les possibilités de la biomanipulation. Ce programme porte sur les recherches sur les biomanipulations en laboratoire, les systèmes à enceintes et les lacs, ainsi que sur les études de modélisation.

Pour des informations supplémentaires, s'adresser à : Convetion Bureau van Namen en Westerlaken, C.P. 1558, 6501 BN Nijmegen, Pays-Bas; téléphone : 31 80 234471.

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Ford, T. E., and R. J. Naiman. Groundwater – surface water relationships in boreal forest watersheds: dissolved organic carbon and inorganic nutrient dynamics

Scarnecchia, D. L., Á. Ísaksson, and S. E. White. Effects of oceanic variations and the West Greenland fishery on age at maturity of Icelandic west coast stocks of Atlantic salmon (*Salmo salar*)

Levine, S. N., and D. W. Schindler. Phosphorus, nitrogen, and carbon dynamics of Experimental Lake 303 during recovery from eutrophication

Hilborn, R., and P. Medley. Tuna purse-seine fishing with fish-aggregating devices (FAD): models of tuna FAD interactions

Latham, K. E., and J. J. Just. Oxygen availability provides a signal for hatching in the rainbow trout (*Salmo gairdneri*) embryo

Lucotte, M. Phosphorus reservoirs in the St. Lawrence upper estuary

Ludwig, D., and C. J. Walters. A robust method for parameter estimation from catch and effort data

Collins, N. Daytime exposure to fish predation for littoral benthic organisms in unproductive lakes

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CJFSDX45(12) 2054–2251 (1988)
ISSN 0706-652X

Subscription rates/Abonnements

Canada: Institutional/Collectif $170; Personal/Personnel $65
Other countries/Autres pays : Institutional/Collectif $204; Personal/Personnel $78
Single issue/Par numéro : $12.95 (Canada); $15.55 (Other countries/Autres pays)